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(54) Title: ANTIVIRAL IMIDAZOLINONE NUCLEOSIDE DERIVATIVES

(57) Abstract

The present invention provides novel nucleoside or nucleotide analogs having a 4-acetylimidazolin-2-one base. The present invention also provides methods for inhibiting virally encoded reverse transcriptases, inhibiting viral replication of those viruses that utilize reverse transcriptase for replication and for treating or preventing diseases caused by viruses whose life cycle requires a reverse transcriptase, e.g., human immunodeficiency viruses, hepatitis B virus, human T cell leukemia/lymphoma viruses, and the like.

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ANTIVIRAL IMIDAZOLINONE NUCLEOSIDE DERIVATIVES

This invention was made in part with United States government support under grant number AI27251 awarded by the National Institutes of Health. The United States government has certain rights in the invention.

FIELD OF THE INVENTION:

The present invention relates to novel nucleoside analogs having a 4-acetylimidazolinone ring in place of the pyrimidine or purine rings present in most natural nucleosides and nucleotides. Such nucleoside analogs can inhibit viral DNA polymerases, particularly viral reverse transcriptases, as well as nucleotide biosynthetic enzymes. Such nucleoside analogs can also inhibit replication of retroviruses and hepatitis B virus. The present invention also contemplates compositions and methods for treating diseases caused by retroviruses and RNA viruses, e.g., acquired immunodeficiency syndrome (AIDS), hepatitis B and T-lymphocytic leukemias and the like.

BACKGROUND OF THE INVENTION:

Many viruses, including all retroviruses and the hepatitis B virus rely upon an RNA-dependent DNA-polymerase, or reverse transcriptase, for replication. For example, even though mature hepatitis B virions contain a DNA genome, this DNA genome is transcribed into full length RNA which is then packaged into an immature core containing an RNA "pre-genome" and hepatitis B reverse transcriptase. The reverse

transcriptase then simultaneously copies and degrades the RNA "pre-genome" to produce a hepatitis B DNA. Therefore replication of both retroviruses and hepatitis B is reverse transcriptase-dependent.

Unlike mammalian DNA polymerases, e.g., nuclear DNA polymerase α or mitochondrial DNA polymerase γ, reverse transcriptase is notoriously error-prone and permits a high degree of mispairing in the production of a new DNA strand. Moreover, unlike mammalian (nuclear) DNA polymerase, reverse transcriptase cannot edit and thus does not repair mismatched bases as DNA synthesis proceeds. Various nucleoside analogs have been designed to inhibit viral DNA synthesis, without adversely affecting normal cellular DNA synthesis.

Nucleoside analogs are structurally related, but not identical, to the nucleosides normally used by cells and microorganisms to synthesize DNA. The degree of structural relatedness between a nucleoside analog and the corresponding normal nucleoside is thought to control the extent of incorporation of the analog by DNA polymerases into DNA; the more structurally similar the analog the greater likelihood of its incorporation into DNA. With regard to replication mediated, e.g., by reverse transcriptase, a nucleoside analog must retain sufficient structural similarity to a normal nucleoside to be recognized and used by the enzyme or such an analog will not be useful for treating diseases caused by retroviruses or hepatitis B.

Nucleoside analogs can inhibit viral DNA synthesis in several ways. A nucleoside analog can be a DNA chain terminator if the 3'-OH normally present on a nucleoside either is not present or has been replaced

with a substituent which cannot form a phosphodiester bond to the next nucleotide in the growing DNA chain. Non-chain terminating analogs, which are incorporated into viral DNA, can lead to viral chromosomal mutation as the analogs are misread in subsequent rounds of DNA synthesis. Moreover, the triphosphate forms of nucleoside analogs can also competitively inhibit reverse transcriptase activity, thereby reducing the amount of viral replication. Nucleoside analogs are therefore useful both as viral mutators and as inhibitors of viral DNA synthesis.

In an ideal situation, cells are unaffected by treatment with nucleoside analogs designed to inhibit viral replication because cellular DNA replication is more precise than replication by reverse transcriptase. For example, cellular enzymes that catalyze DNA replication not only discriminate between nucleosides to a greater degree than reverse transcriptase, but also proofread the incorporated nucleoside for correct base pairing.

However, side effects are frequently observed in patients treated with known nucleoside analogs as a result of incorporation of a nucleoside analog into cellular chromosomes or mitochondrial DNA. Nucleoside analogs which are readily incorporated into chromosomal and mitochondrial DNA are of limited value in the treatment of viral infections. In practice, a nucleoside analog can also inhibit the conversion of normal nucleosides into the nucleoside triphosphate used to synthesize DNA. While inhibition of triphosphate formation can slow the rate of viral replication, inhibition of normal nucleoside triphosphate formation

can also have detrimental effects upon the cell. While a number of nucleoside analogs are known to inhibit viral replication, these analogs cause side effects such as anemia, neutropenia, neuropathy, pancreatitis and other problems (see Saunders et al. 1992 DN&P 5:153-169 for a review).

The first nucleoside analog reported to have activity against human immunodeficiency virus (HIV) in vitro was 3'-azido-3'-deoxythymidine (AZT) (Mitsuya et al. 1985 Proc. Natl. Acad. Sci. USA 82: 7096-7100). AZT is both a competitive inhibitor of reverse transcriptase and a DNA chain terminator. AZT also exhibited beneficial effects in clinical trials (Fischl et al. 1987 N. Eng. J. Med. 317: 185-191) and was the first drug approved by the Food and Drug Administration for treatment of AIDS. AZT can penetrate the blood-brain barrier and therefore has efficacy against HIV caused dementia. However the serum half-life of AZT is only about 1.1 hours and its major metabolite is an inactive 5'-glucuronide (Yarchoan et al. 1989 N. Eng. J. Med. 321: 726-738).

While AZT has proven efficacy against HIV, considerable toxicity problems have been encountered as a result of AZT therapy. The most serious of these has been suppression of bone marrow formation resulting in anemia and neutropenia (Richman et al. 1987 N. Eng. J. Med. 317: 192-197). Bone marrow formation is thought to be suppressed because AZT is toxic to bone marrow progenitor cells (Sommadossi et al. 1987 Antimicrob. Agents Chemotherapy 31: 452-454). The effect of AZT on bone marrow is sufficiently severe to necessitate blood transfusion, AZT dose reduction or even cessation of AZT

treatment. Moreover, AZT-resistant strains of HIV have developed in patients receiving AZT therapy (Larder et al. 1989 Science 243: 1731-1734). Clearly, improved non-AZT therapeutic agents are needed for treatment of HIV infection.

Several 2',3'-dideoxy (dd) nucleosides were subsequently tested in vitro for efficacy against HIV including, for example, dideoxycytidine (ddC), dideoxyadenine (ddA) and dideoxyinosine (ddI) (Mitsuya et al. 1986 Proc. Natl. Acad. Sci. USA 83: 1911-1915).

The ddC analog was the first, after AZT, to be evaluated clinically. This analog is not readily metabolized to an inactive form and is quite stable in plasma. In clinical trials ddC has provided evidence of activity against HIV (Yarchoan et al. 1988 Lancet i:76-81; and Merigan et al. 1989 Ann. Intern. Med. 110: 189-194). Moreover, ddC has good bioavailability after oral administration. However, ddC causes peripheral neuropathy, possibly because ddC may inhibit mitochondrial DNA synthesis, and ddC is a potent inhibitor of mammalian nuclear DNA polymerase.

The ddT analog has only weak activity against HIV and has not been further developed as an anti-retroviral agent.

ddA and ddI are both converted to an active ddATP species. Although ddATP is less potent than the AZT triphosphate (AZTTP) or ddCTP, the intracellular half-life of ddATP is 12 hr, at least 4-fold longer than AZTTP and ddCTP. However, both ddA and ddI are highly susceptible to solvolysis of the glycosidic linkage which liberates the free purine base. The free base of ddI, hypoxanthine, is less toxic than the free base of

ddA, adenine, which has been shown to cause renal damage (Lindbald et al. 1973 Acta Pharmacol. Toxicol. 32: 246-256). Accordingly, ddI has been pursued in clinical trials over ddA as a therapeutic agent. While ddI does not cause the severe anemia cause by AZT, ddI does have its own side effects: neuropathy and pancreatitis.

Nucleoside analogs have also been developed which have a variety of 3'-substituents, other than the azide on AZT, and in place of the 3'-OH present on naturally occurring nucleosides. For example a 3'-fluoro analog of thymidine (FDT) has been developed which has potent in vitro activity against HIV; however, initial studies indicate that this analog can be toxic and therefore would have no advantage over AZT (Mansuri et al. 1990 Antimicrob. Agents Chemother. 34: 637-641).

Uridine and cytidine analogs of AZT, 3'-azidodideoxyuridine (AZU) and 3'-azidodideoxycytidine, have also been developed (Eriksson et al. 1989 Antimicrob. Agents Chemother. 33: 1729-1734; and Chu et al. 1989 J. Med. Chem. 32: 612-617). However, these analogs do not appear to be as effective as AZT in vitro. Clinical trials have yet to be completed on these analogs.

Several 2',3'-dideoxy-2',3'-didehydronucleoside analogs, commonly referred to as d4 compounds, have been made and preliminarily tested. The most notable d4 analogs are d4C and d4T (Mansuri et al. 1990; Balzarini et al. 1986 Biochem. Biophys. Res. Commun. 140: 735-42; and Ho et al. 1989 Antimicrob. Agents Chemother. 33: 844-849). In vitro studies of d4T indicate that this analog may be less toxic than AZT and may act more selectively on reverse transcriptase than

AZT (Mansuri et al. 1990). However, d4T treatment, similar to treatment with many of the nucleoside analogs described above, causes peripheral neuropathy.

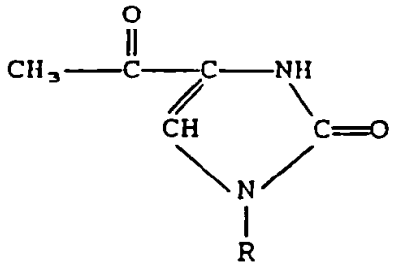
Accordingly, there is a long-standing need for effective, non-toxic agents to treat HIV infections and other infections caused by organisms whose replication depends upon reverse transcriptase, notably hepatitis B, human T-cell lymphotropic virus-I and -II (HTLV-I and HTLV-II) and the like.

The present invention is directed to novel nucleoside analogs having a 4-acetylimidazolinone ring, as well as to methods of using such nucleoside analogs for inhibiting reverse transcriptase, viral replication and diseases caused by retroviruses and hepatitis B. Imidazolinone nucleosides have been synthesized (Otter et al. 1969 J. Org. Chem. 34: 2636-2642). However these synthetic procedures did not yield the specific compounds contemplated herein. The present analogs are more selective for reverse transcriptase than known nucleoside analogs and are not substantially incorporated into cellular or mitochondrial DNA. Therefore, the present analogs do not exhibit many of the toxicity problems associated with known nucleoside analogs.

SUMMARY OF THE INVENTION:

The present invention provides novel nucleoside or nucleotide analogs having a 4-acetylimidazolin-2-one base.

One embodiment the present invention provides a compound of the following formula:



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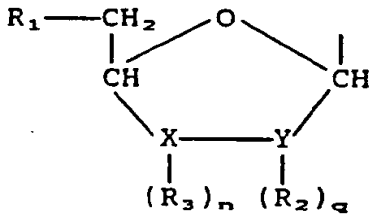
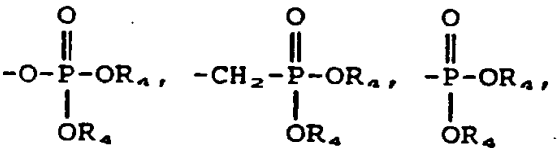
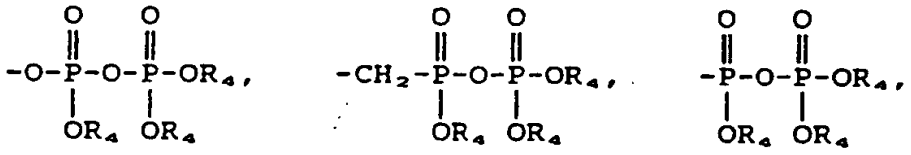
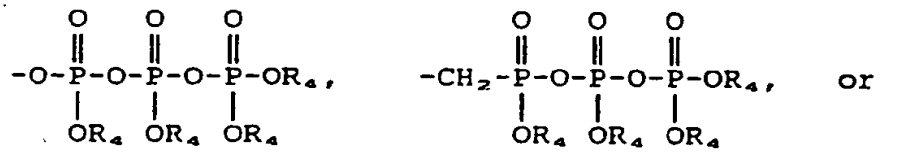
wherein R is hydrogen or



wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, 





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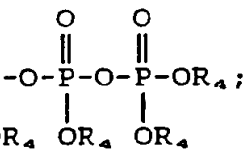


R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

X and Y each are independently -CH-, -O-, -S-, or X and Y together are -C=C-;

R_2 is hydrogen, lower alkoxy or hydroxy;

R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido;

n and q are independently 0 or 1;

when X is -O- or -S- then n is zero;

when Y is -O- or -S- then q is zero; or

a pharmaceutically acceptable salt thereof.

The present invention further provides a method of inhibiting DNA synthesis catalyzed by reverse transcriptase which includes contacting the reverse transcriptase with a reverse transcriptase inhibiting amount of at least one nucleoside or a nucleotide analog having a 4-acetylimidazolin-2-one base.

The present invention is still further directed to a method of inhibiting retroviral replication which includes contacting a retrovirus with at least one retrovirus replication-inhibiting amount of a nucleoside or a nucleotide analog having a 4-acetylimidazolin-2-one base.

The present invention also provides a method of treating or preventing animal retroviral infection which includes administering to an animal an anti-

retroviral effective amount of at least one nucleoside analog or a nucleotide analog having a 4-acetylimidazolin-2-one base.

The present invention further provides a method of treating or preventing human hepatitis B infection which includes administering to a patient an anti-hepatitis B effective amount of at least one nucleoside or a nucleotide analog having a 4-acetylimidazolin-2-one base.

In an additional embodiment the present invention is directed to a method of treating or preventing human immunodeficiency virus (HIV) infection which includes administering to a patient an anti-HIV effective amount of at least one nucleoside or a nucleotide analog having a 4-acetylimidazolin-2-one base.

BRIEF DESCRIPTIONS OF THE DRAWINGS:

Fig. 1 depicts the structures of deoxythymidine and 1-(ß-D-2-deoxyribofuranosyl)-4-acetylimidazolin-2-one (abbreviated deoxyimidine or dImd). X-ray diffraction analysis of an deoxyimidine 4-methoxycarbonyl derivative (i.e., 1-(ß-D-2-deoxyribofuranosyl)-4-methoxycarbonylimidazolin-2-one) indicates that the carbonyl oxygen of the imidine base is correctly oriented to permit internucleotide hydrogen bonding (base pairing).

Fig. 2 illustrates the close structural similarity between the energy minimized structures of thymidine (dThd) and 1-(ß-D-2-deoxyribofuranosyl)-4-acetylimidazolin-2-one (dImd).

Fig. 3 provides a comparison of the distances and angles of NH-O and N-HN hydrogen bonds in an adenine-thymine (A==T) and an adenine-imidine (A==Im) base pair.

Fig. 4 provides a graph comparing the percent inhibition of human immunodeficiency virus reverse transcriptase (HIV-RT, filled circles) with the percent inhibition of a nuclear human DNA polymerase α from MOLT-4 human lymphocytes (MOLT-4 POLY α, filled diamonds) at various concentrations of 1-(ß-D-2-deoxyribofuranosyl)-4-acetylimidazolin-2-one 5'-triphosphate (dImdTP). The molar concentration of dImdTP required for 50% inhibition (IC_{50}) of both HIV-RT (38 nM) and MOLT-4 POLY α (17 µM) is also provided. As illustrated, about 500-fold less dImdTP is required to inhibit HIV-RT than MOLT-4 POLY α.

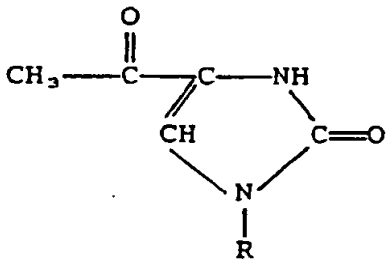
Fig. 5 depicts the percent inhibition of HIV reverse transcriptase during synthesis of a poly(dG) strand on a poly(dC) template when varying concentrations of 1-(ß-D-2-deoxyribofuranosyl)-4-acetylimidazolin-2-one 5'-triphosphate (dImdTP) or dTTP are present. As illustrated, dImdTP (filled circles) is a much more effective competitive inhibitor of HIV reverse transcriptase than is dTTP (filled diamonds).

Fig. 6 depicts the percent protection afforded to HIV infected cells by varying concentrations of 1-(ß-D-2-deoxyribofuranosyl)-4-acetylimidazolin-2-one (dImd). The percent protection (solid line ——) was defined as the percent viable HIV-infected cells relative to uninfected dImd-treated cells. The cytopathic effect of HIV on untreated cells is provided for comparison (dotted line .....). Fig. 6 also depicts the cytoxicity

of dImd (broken line ----), defined as the percent viable non-infected cells treated with dImd. Little or no cytoxicity was observed for dImd concentrations up to 1 mM. A 50% protection reference line is also provided (-•-•-•-•-).

DETAILED DESCRIPTION OF THE INVENTION:

The present invention relates to novel nucleoside or nucleotide analogs having a 4-acetylimidazolin-2-one ring in place of the pyrimidine rings found in naturally occurring nucleosides. As described herein these analogs have utility for inhibiting reverse transcriptase, retroviral replication and hepatitis B replication. In a preferred embodiment the present analogs can be used for inhibiting the replication of human immunodeficiency virus, as well as for treating or preventing human immunodeficiency viral infections. 4-Acetyl-2-imidazolinone compounds of the present invention are of the formula:



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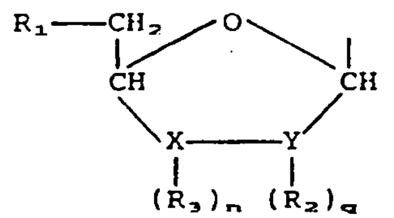
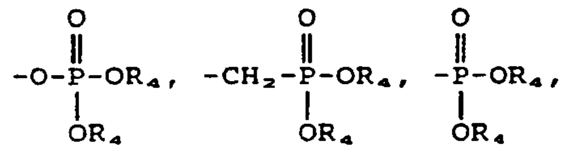
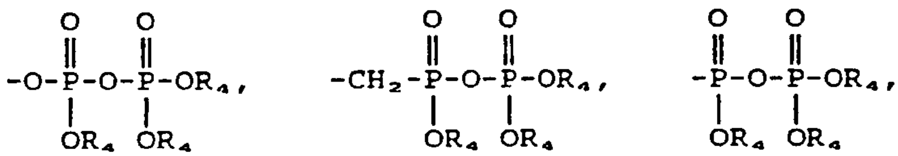
wherein R is hydrogen or

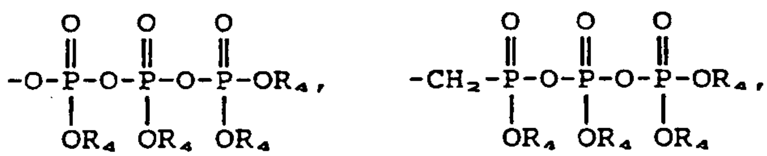


wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate,







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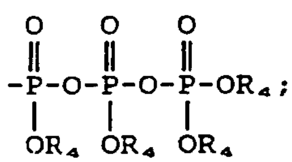


R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

X and Y each are independently $-\underset{|}{CH}-$, -O-, -S-, or X and Y together are -C═C-;

R_2 is hydrogen, lower alkoxy or hydroxy;

R_3 is hydrogen, lower alkoxy, hydroxy, halo or azido;

n and q are independently 0 or 1;

when X is -O- or -S- then n is zero;

when Y is -O- or -S- then q is zero; or

a pharmaceutically acceptable salt thereof.

As used herein a 4-acetyl-2-imidazolinone nucleoside having a deoxyribose sugar is referred to as imidine or dImd; the 4-acetyl-2-imidazolinone base is abbreviated as Im.

As provided herein, a lower alkyl, singly or in combination with other groups, contains up to six carbon atoms in the main chain and a total of 10 carbon atoms if the alkyl is branched. Lower alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, t-butyl, sec-butyl, isobutyl, amyl, isoamyl, pentyl, isopentyl, hexyl and the like. Methyl and ethyl groups may be abbreviated herein as Me and Et, respectively. The preferred lower alkyl groups contain one to four carbon atoms.

A lower alkoxy substituent is a lower alkyl covalently attached via an oxygen atom, i.e., -O-lower alkyl. A lower alkanoyl substituent is a lower alkyl containing a carbonyl group.

As used herein an acyloxymethyl group is a lower alkyl group covalently attached to a $-CO-O-CH_2-$ group, i.e., a lower alkyl-$CO-O-CH_2-$.

An azido group is an $-N_3$ group.

Halogen or halo groups include fluoro (-F), chloro (-Cl), bromo (-Br) and iodo (-I). Fluoro is a preferred halo group.

The term aryl refers to an aromatic moiety containing 6-10 ring carbon atoms and includes phenyl, α-naphthyl, ß-naphthyl, and the like. An aryl-lower alkyl refers to an aryl group with one or more lower alkyl substituents.

A sulfonate ester is a $-OSO_2-$ group; and a sulfinate ester is an -SO-O- group. A lower alkyl sulfonate ester is a $-OSO_2$-lower alkyl and a lower alkyl sulfinate ester is a -SO-O-lower alkyl. An arysulfonate ester is a $-OSO_2$-aryl and an arylsulfinate ester is a -SO-O-aryl wherein the aryl may be substituted with 1-3 lower alkyl groups, 1-2 halogens or 1-2 nitro group.

As described, the X and Y ribose ring atoms are independently >CH, -O-, -S-, or X and Y can be taken together to form -C=C-. In a preferred embodiment X is >CH, -O- or X is taken together with Y to form -C=C-. Preferred Y substituents are >CH, or Y is taken together with X to form -C=C-. When X and Y are taken together to form -C=C- then a partially unsaturated ribose ring is present.

The subscripts, n and q define the number of R_3 and R_2 groups, respectively, wherein n and q are independently 0 or 1. As defined, n is 0 when X is -O- or -S-. Therefore, R_3 is not present when X is -O- or -S- and can only be present when X is >CH-. Similarly, q is 0 when Y is -O- or -S- and there are no R_2 substituents when Y is -O- or -S-. Therefore, R_2 can only be present when Y is >CH-.

In a preferred embodiment n and q are both 1.

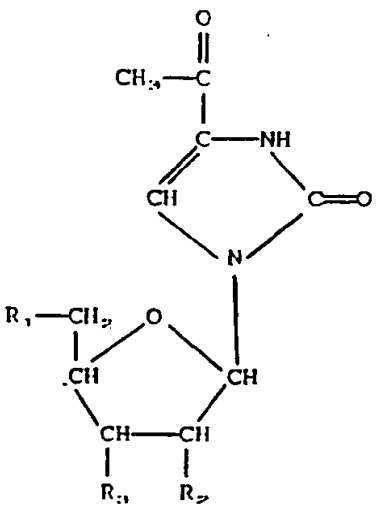
R_3 can be hydrogen, lower alkoxy, hydroxy, halo or azido. A preferred halo group for R_3 is fluoro. Preferably, R_3 is hydrogen, hydroxy, fluoro or azido.

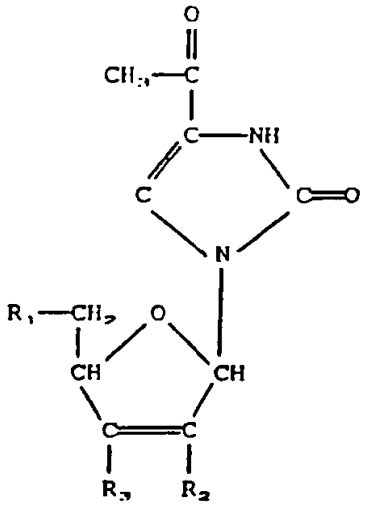
Analogs of the present invention where R_3 is hydrogen, lower alkoxy, halo or azide can terminate a growing DNA strand, i.e., can cause chain termination, because such substituents cannot form a bond to the 5'-phosphate of another nucleotide. However, analogs of the present invention where R_2 is hydroxy are non-chain terminating since such a hydroxy group can bond with a 5'-phosphate of another nucleotide.

R_2 can be hydrogen, lower alkyl, hydroxy, lower alkoxy. In a preferred embodiment R_2 is hydrogen, i.e., the present nucleoside analogs preferably contain 2'deoxyribose or a 2'-deoxyribose with one of the present X or R_3 substituents.

As provided herein, R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, phosphorylphosphonate, pyrophosphorylphosphonate and the like. R_1 moieties can have an R_4 substituent attached to a phosphate oxygen or a phosphonate oxygen. The R_4 group can be hydrogen, cation, lower alkyl, acyloxymethyl and the like. Such cations include Na^+, K^+, Li^+, Ca^{++}, Mg^{++}, Ba^{++}, NH_4^+, monoethanolammonium, tri-cyclohexylammonium, and the like.

Preferably, the nucleoside analogs of the present invention have X and Y as CH, or X and Y are taken together to form C=C. In another preferred embodiment X is -O- and Y is -CH-. Therefore, the present compounds are preferably of the formula:





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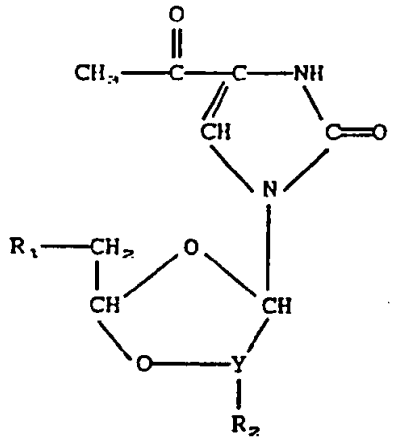


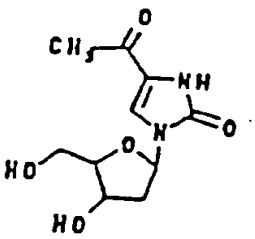
wherein n and q are both 1 and Y, R_1, R_2 and R_3 are as defined hereinabove.

Each of the present analogs can have a 5'-hydroxy, a 5'-monophosphate, a 5'-diphosphate or a 5'-triphosphate or a derivatized mono-, di- or tri-phosphate. Preferred nucleoside analogs have a 5'-hydroxy, a 5'-monophosphate, a 5'-phosphonate or a 5'-triphosphate, i.e., R_1 is preferably -OH, $-OPO_3^-$,

$-CH_2-PO_3^-$, $-PO_3^-$ or $-O-PO_2^--OPO_2^--OPO_3^-$. These preferred phosphate groups can also have a proton or cation associated with one or more phosphate oxygens; when such a cation is present a pharmaceutically acceptable salt can form.

More preferred nucleoside analogs of the present invention are depicted below.

II



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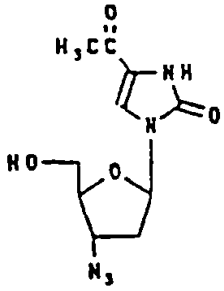

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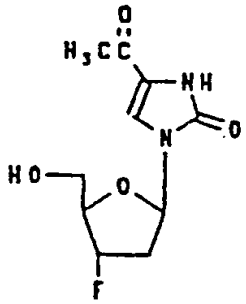
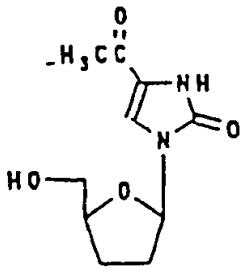
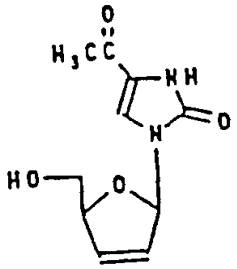
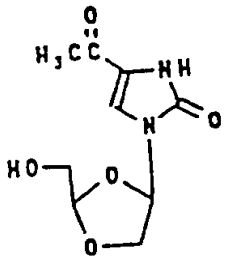




V



VI



VII

The novel compounds of the present invention were designed with consideration for the size and geometry of the 4-acetylimidazolinone base as relating to normal nucleoside bases, e.g. thymine, and the base pairing properties thereof. Careful molecular modeling indicated that 4-acetylimidazolinone deoxyriboside (dImd) was an excellent structural match for thymidine (dT) and could mimic the base pairing of thymine (T) and, to a lesser degree, cytosine (C).

The remarkable similarities between the energy minimized structures of thymidine and 1-(β-D-2-deoxyribofuranosyl)-4-acetylimidazolin-2-one (referred to herein as dImd) are apparent from a review of Figs. 1 and 2. In comparison to the 5-methyl-2,4-dioxopyrimidine ring of thymidine, the present analogs have a 4-acetylimidazolinone ring. The carbonyl of the 4-acetyl group of the present 4-acetylimidazolinone analogs can assume the role of the 4-oxo group of thymidine. Similarly the methyl within the present 4-acetyl group corresponds to the 5-methyl on thymidine.

The structures of the present novel nucleotide analogs are similar enough to normal nucleotides, e.g., thymidine 5'-triphosphate, that they are readily recognized by and fit into the active site of reverse transcriptase. However nuclear and mitochondrial mammalian DNA polymerases, which are much more discriminating than reverse transcriptase, can detect the structural differences between these analogs and natural nucleotides, and do not as readily recognize or bind these analogs.

While the present analogs are recognized and bound by reverse transcriptase, these analogs are also sufficiently different from normal nucleotides to act as competitive inhibitors of the enzyme. In other words, the present analogs can be bound within the active site of reverse transcriptase but are only slowly released and replaced by an incoming normal nucleotide. For example, as illustrated in Fig. 4, as little as 38 nanomolar of a representative analog of the present invention, 1-(2-Deoxy-ß-D-ribofuranosyl)-4-acetylimidazolin-2-one 5'-triphosphate (dImdTP), can strongly inhibit incorporation of thymidine into DNA synthesized by HIV reverse transcriptase. Since dImdTP has a free 3'-OH which is available for chain elongation, dImdTP does not inhibit DNA synthesis by chain termination; rather dImdTP acts as a competitive inhibitor of reverse transcriptase. Moreover, under similar conditions, thymidine incorporation into DNA synthesized by a human nuclear DNA polymerase is not detectably inhibited by dImdTP. Inhibition of a mammalian nuclear DNA polymerase required at least a 500-fold greater concentration of dImdTP than was

required for inhibition of reverse transcriptase (Fig. 4).

X-ray diffractional analysis of the 4-methoxycarbonyl imidazolinone derivative (Fig. 1) of the present 4-acetylimidazolinone compounds demonstrated that the carbonyl of the 4-acetyl group is appropriately oriented to correctly base pair with adenine (A).

Molecular modeling further revealed a remarkable similarity between the adenine:imidine (A:Im) base pair and the adenine:thymine (A:T) base pair. The distances between the hydrogen bonding electronegative atoms of a A:Im base pair and a A:T base pair are very similar (Fig. 3).

However, the angle of hydrogen bonding in the A:Im base pair differs from that in the A:T base pair, by about 15° to 20° (Fig. 3). This difference in bonding angles can weaken the base pairing between adenine and imidine resulting in recognition and replacement of imidine with thymine by nuclear or mitochondrial DNA polymerases.

The structural similarities between thymidine and the present 4-acetylimidazolinone analogs, therefore, allow incorporation of these analogs into DNA synthesized by reverse transcriptase, e.g., to form dA:dImd in place of dA:dT. The slight dissimilarities between the base pairing of thymine and of imidine, however, permit nuclear and mitochondrial mammalian DNA polymerases to recognize and correct a dA:dImd base pair. Therefore, the present analogs are not substantially incorporated into mammalian DNA, not only because mammalian DNA polymerases do not initially bind the free analog to any great extent, but also because

mammalian DNA polymerases can recognize and remove a rarely incorporated and improperly base paired analog.

The small structural and base pairing differences between the present analogs and normal nucleotides can lead to misreading of an analog incorporated into viral DNA by reverse transcriptase in a subsequent round of DNA replication. Such misreading can result an A→G transition mutation. This occurs as reverse transcriptase incorporates Im opposite to A and then in the next round of synthesis mistakenly pairs the Im with guanine to form a G:Im base pair. The next round of replication readily converts the G:Im base pair to a G:C base pair. Accordingly the present 4-acetylimidazolinone nucleoside analogs can cause mutations of viral DNA synthesized by reverse transcriptase which can cripple or kill the virus, e.g., a retrovirus or hepatitis B virus.

By replacing the 3'OH of the present analogs with a group that cannot form a covalent linkage to a nucleoside 5'-monophosphate, such analogs can also be chain terminators for DNA synthesized by reverse transcriptase. For example, the present analogs can be chain terminators when the 3'-OH is replaced with a hydrogen, an azido, a halo and the like. Therefore, in addition to causing mutations in the viral genome, the present analogs can actually inhibit DNA replication catalyzed by reverse transcriptase in at least two different ways, i.e., by competitive inhibition and by chain termination. This three-fold effect of the present analogs upon retroviruses and hepatitis B, i.e., mutation of viral genomes, competitive inhibition of viral DNA synthesis and chain termination of newly

replicated viral DNA, makes these analogs extremely effective anti-viral agents.

Moreover the multi-fold effects of these analogs on reverse transcriptase means that viruses can not readily develop resistance against the present analogs. Accordingly the present analogs can have efficacy against drug-resistant viral strains.

Moreover, the present 4-acetylimidazolinone analogs are more selective for reverse transcriptase than known nucleoside analogs which contain normal pyrimidine or purine bases, e.g. thymidine analogs AZT, d4T, ddT, DFT and the like. This higher selectivity for reverse transcriptase, over mammalian DNA polymerases, makes the present 4-acetylimidazolinone analogs less toxic and therefore, more efficacious than known nucleoside analogs.

The present invention thus contemplates a method of inhibiting DNA synthesis catalyzed by reverse transcriptase which includes contacting the reverse transcriptase with a reverse transcriptase-inhibiting amount of at least one nucleoside or nucleotide analog of the present invention.

The present invention also provides a method of inhibiting viral replication catalyzed by reverse transcriptase which includes contacting a virus with a reverse transcriptase-inhibiting amount of at least one nucleoside or a nucleotide analog of the present invention. Alternatively the virus can be contacted with a viral-replication inhibiting amount of at least one of the present analogs.

In another embodiment the present invention is directed to a method of treating or preventing

retroviral infection in an animal which includes administering to an animal an anti-retroviral effective amount of a compound of the present invention. Such an anti-retroviral effective amount can be an amount sufficient to inhibit retroviral replication.

As contemplated herein, the present nucleoside analogs can be used alone or in combination with other therapeutic agents to inhibit DNA synthesis catalyzed by reverse transcriptase, to inhibit viral replication catalyzed by reverse transcriptase and to treat or prevent retroviral or hepatitis B infection.

As used herein treating retroviral infections means to slow the progress of the disease, to ameliorate symptoms of such infections which are already visible and to preclude or diminish the onset of new symptoms. Preventing animal retroviral infections refers to delaying or preventing the onset of initial symptoms of the infection.

Retroviral infections which can be treated or prevented by administration of the nucleoside analogs of the present invention include infections caused by a lentivirus, oncovirus C, oncovirus A, oncovirus B, cisternavirus, Spumavirus F and the like. For example, the present compounds have efficacy against human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), human intracisternal retrovirus, human T cell leukemia/lymphoma virus type I (HTLV-I), human T cell leukemia/lymphoma virus type II (HTLV-II), Spumavirus F foamy virus, mouse mammary tumor virus-S (MMTV-S or Bittner's virus), mouse mammary tumor virus-P (MMTV-P or GR virus), mouse mammary tumor virus-L (MMTV-L), Rous sarcoma virus (RSV), Rous-associated

viruses (RAV), related chicken sarcoma viruses, avian leukosis viruses (ALV), reticuloendotheliosis viruses, pheasant viruses, murine sarcoma viruses (MSV), murine leukosis virus G (Gross or AKR virus), murine leukosis virus-Friend (MLV-F), murine leukosis virus-Moloney (MLV-M), murine leukosis virus-Rauscher (MLV-R), murine radiation leukemia virus, murine endogenous viruses, rat leukosis virus, feline immunodeficiency virus, feline leukosis viruses, feline sarcoma virus, feline endogenous virus (RD114), hamster leukosis virus, porcine leukosis virus, bovine leukosis virus, simian immunodeficiency virus, primate sarcoma viruses (woolly monkey; gibbon ape), primate sarcoma-associated virus, primate endogenous viruses including baboon endogenous virus (BaEV), stumptail monkey virus (MAC-1), owl monkey virus (OMC-1), viper virus, mason-pfizer monkey virus (MPMV), langur virus, squirrel monkey virus, visna virus of sheep, caprine arthritis-encephalitis virus, equine infectious anemia, and the like.

In a preferred embodiment the nucleoside analogs of the present invention are used to prevent infections caused by a lentivirus, an oncovirus C and the like, e.g., human immunodeficiency virus-1 (HIV-1), human immunodeficiency virus-2 (HIV-2), human intracisternal retrovirus, human T cell leukemia/lymphoma virus type I (HTLV-I), human T cell leukemia/lymphoma virus type II (HTLV-II), feline immunodeficiency virus, simian immunodeficiency virus, visna virus of sheep, caprine arthritis-encephalitis virus, equine infectious anemia, and the like.

In an especially preferred embodiment the analogs of the present invention can be used to prevent

or treat a human immunodeficiency virus infection, i.e., infections caused by HIV-1, HIV-2 and the like.

Moreover the present methods can be used in a method of treating or preventing hepatitis B infection in a patient which includes administering to a patient an anti-hepatitis B effective amount of a compound of the present invention. While hepatitis B is not classified as a retrovirus, the replication cycle of hepatitis B requires a virally-encoded reverse transcriptase. Therefore, the present compounds have utility for inhibiting hepatitis B virus replication and for treating and preventing hepatitis B infections.

Reverse transcriptases which can be inhibited by the present methods also include any reverse transcriptase of the foregoing retroviral and hepatitis B viral species. The present methods are preferably employed to inhibit the reverse transcriptases of HIV-1, HIV-2, HTLV-I, HTLV-II, hepatitis B and the like.

As used herein an amount of the novel nucleoside analogs of the present invention which is sufficient to inhibit reverse transcriptase (i.e. a reverse transcriptase-inhibiting amount) is an amount which detectably inhibits the synthesis of DNA by reverse transcriptase. The amount of DNA synthesized by reverse transcriptase in the presence of varying amounts of the present compounds can be observed by any procedure known in the art. For example, procedures for observing the amount of DNA synthesized either in vivo or in vitro by reverse transcriptase are provided in Eriksson et al. (1989 Antimicrobial Agents and Chemotherapy 33: 663-669); Bardos et al. (1992 Antimicrob. Agents and Chemotherapy 36: 108-114). Such

methods can involve the use of a detectable reporter molecule which is covalently attached to a nucleotide. This labeled nucleotide is provided to the reverse transcriptase under the conditions where inhibition is to be effected, e.g., either in vivo or in vitro. After permitting DNA synthesis to occur for the desired length of time, the DNA synthesized by reverse transcriptase can be separated from the unincorporated labeled nucleotide and the amount of reporter molecule present in such DNA is measured. A decrease in the amount of reporter molecule present in DNA synthesized in the presence of the analogs of the present invention, as compared to the amount synthesized in the absence of an analog, indicates that inhibition has occurred.

A "reporter molecule", as used herein, is a molecule which, by its chemical nature, provides an analytically identifiable signal allowing detection of an incorporated nucleotide. Detection is preferably quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclides covalently linked to nucleotides which are incorporated into DNA synthesized by reverse transcriptase or by mammalian DNA polymerases. Commonly used enzymes include horseradish peroxidase, alkaline phosphatase, glucose oxidase and ß-galactosidase, among others. The substrates used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for horseradish peroxidase, 1,2-phenylenediamine, 5-aminosalicyclic acid or tolidine are commonly used.

Sambrook et al. 1989, Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Laboratory Press) provide a review of many useful procedures for observing the incorporation of a reporter molecule into DNA.

As used herein, a virus replication-inhibiting amount of the present compounds is an amount sufficient to detectably reduce the rate of virus replication catalyzed by reverse transcriptase. Such an amount can also inhibit reproduction of a retrovirus or hepatitis B virus. For example, the rate of viral reproduction can be determined by observing the number of viruses or the amount of viral antigen (e.g., p24 of HIV), the amount of reverse transcriptase activity or the amount of viral nucleic acid present over time. The detection of antibodies in animal body fluids (e.g., serum, urine and the like) which react with viral antigens is also diagnostic of viral infection and viral replication.

Procedures for detecting and quantitating viruses both in vitro and in vivo are available, e.g., Agrawal et al. (1988 Proc. Natl. Acad. Sci. USA 85: 7079-7083); Balzarini et al. (1991 AIDS 5: 21-28); Balzarini et al. (1988 Biochem. Pharmacol. 37: 2847-2856); Goodchild et al. (1988 Proc. Natl. Acad. Sci. USA 85: 5507-5511); Weislow et al. (1989 J. Natl. Cancer Inst. 81: 577-586); Zamecnik et al. (1978 Proc. Natl. Acad. Sci. USA 75: 280-284) provide useful procedures.

An anti-retroviral effective amount is an amount of at least one of the present analogs which detectably reduces the number of infective retroviruses, the retroviral infectivity, the symptoms or progression of a retroviral infection. Procedures for determining the number or infectivity of retroviruses are known and

readily available to the skilled artisan as described hereinabove. Moreover the symptoms associated with retroviral disease are well documented and can be used to assess the progression of the disease (e.g., see Wilson et al. 1991 Harrison's Principles of Internal Medicine, twelfth edition, McGraw-Hill, Inc., New York; Centers for Disease Control 1986 Morb. Mort. Week Rep. 35:334; Centers for Disease Control 1987 Morb. Mort. Week Rep. 36:1S; Centers for Disease Control 1989 Morb. Mort. Week Rep. 38:S-6).

An anti-HIV effective amount is an amount of at least one of the present analogs sufficient to inhibit or reduce the replication of HIV DNA, the amount of HIV antigen, the number of HIV-induced syncytia, the number of infective HIV virions, the HIV infectivity or the progression of a HIV infection. The amount of DNA replicated by HIV can be measured in vivo or in vitro. Measurements of the amount of HIV DNA replicated include enzymatic assays, e.g., as described in Eriksson et al. (1989), cell culture measurements, e.g., as described in Weislow et al. and the like. The amount of HIV antigen can be routinely detected by the skilled artisan in patient body fluids, e.g., blood (serum), urine and the like. Commonly available procedures for HIV antigen detection include enzyme-linked immunosorbant assays (ELISA), Western analyses, immunofluorescence assays, radioimmunoprecipitation assays and the like. The number of infective HIV virions can be assayed e.g., as described in Balzarini et al. (1991 AIDS 5: 21-28) and Balzarini et al. (1988 Biochem. Pharmacol. 37: 2847-2856). The progression of HIV infection in humans is well documented (e.g., Wilson et al. 1991; Centers for

Disease Control 1986; Centers for Disease Control 1987; Centers for Disease Control 1989).

An anti-hepatitis B effective amount is an amount of the present analogs which detectably reduces the amount of hepatitis B antigens observed, the anti-hepatitis B antibody titer in a host serum sample, the number of infective hepatitis B viruses, the hepatitis B infectivity or the progression of a hepatitis B infection. Hepatitis B antigens which can be detected by routine procedures available to the skilled artisan include hepatitis B surface antigen, hepatitis B core antigen, hepatitis B e antigen, and the like. For example, the number and infectivity of hepatitis B virions can be detected by cell culture assay and by observing the number of Dane particles or the number of large 42 nm spherical intact hepatitis B virions in a given volume, e.g., the number of intact virions in a blood sample.

Preferably a reverse transcriptase inhibiting amount, a retrovirus replication-inhibiting amount, an anti-retroviral effective amount, an anti-HIV effective amount and an anti-hepatitis B effective amount does not substantially inhibit DNA synthesis catalyzed by nuclear or mitochondrial DNA polymerase. Moreover such amounts preferably inhibit DNA synthesis mediated by reverse transcriptase by at least about 50% to at least about 80%, and more preferably by at least 90%. Preferred dosages for compositions comprising the present compounds are provided below.

The compounds of the present invention can be prepared by art recognized techniques using protecting groups, leaving groups, activating groups and the like

as needed. Starting compounds can be chosen which have X, Y, R_2 and R_3 groups in the desired positions. Alternatively, a leaving group may be used in the desired R_2 or R_3 position, and the appropriate R_2 or R_3 group may replace the leaving group in a later synthetic step. Another alternative is to employ a protecting group on a reactive group which may be present on starting materials, e.g., an amine, amide, carboxylate, hydroxy or similar reactive group on the chosen starting material. The use of leaving or protecting groups prevents undesirable side reactions from occurring, while permitting desired reactions to take place.

As is generally known in the art, and for the purposes of the present invention, a leaving group is defined as a group which is readily broken away from its union with a carbon atom. These groups are readily recognizable by one skilled in the art. Suitable leaving groups are generally electron attracting groups, either because of their electronegativity or because they have an inductive effect, and may include groups such as halides, N_3, HO-Aryl, or HSO_3-Aryl groups, and the like.

A protecting group is covalently bound to a reactive group to render the reactive group unreactive while allowing desired reactions to take place. To be useful, a protecting group must in addition be easily removed without chemically altering the remainder of the molecule, and must regenerate the correct structure of the reactive group. Examples of protecting groups effective with, for example, primary and secondary amino groups include acetyl, carbobenzoxy (cleaved by catalytic hydrogenation), tert-butoxycarbonyl (cleaved

by mild acid treatment) and 9-fluorenylmethoxycarbonyl (cleaved by secondary amines). Alcohols may be protected with, for example, trityl, mesyl, benzoyl or acetyl blocking groups. Carboxylates can be protected by ester groups. A comprehensive review of useful protecting groups is provided in Greene, 1981 Protective Groups in Organic Synthesis (John Wiley & Sons, New York), the contents of which are herein incorporated by reference.

As used herein, an activating group is a group which, when bound to an oxygen, facilitates cleavage and removal of the oxygen from the present nucleoside analogs. Activating groups contemplated by the present invention include lower alkyl sulfonate, arylsulfonate, trifluoromethylene, cyano, fluoroalkylsulfonate, aryloxy and the like. Such a lower alkyl sulfonate can be a methyl sulfonate (i.e., mesylate), ethyl sulfonate, ammonio-alkylsulfonate (i.e., betylate) and the like. An arylsulfonate can be a tolylsulfonate (i.e., tosylate), bromophenylsulfonate (i.e., brosylate), nitrophenyl-sulfonate (i.e., nosylate) and the like. As used herein lower fluoroalkylsulfonate includes a trifluoromethylsulfonate (i.e., lower alkyl-OSO_2CF_3 or triflate), a nonafluorobutyl-sulfonate (i.e., lower alkyl-OSO_2-C_4F_9 or nonaflate), a 2,2,2-trifluoroethylsulfonate (i.e., lower alkyl-OSO_2-CH_2-CH_2CF_3 or tresylate) and the like. Preferred activating groups are lower alkyl sulfonic esters. More preferred activating groups are mesylates.

Prior to attachment of the activating group, a leaving group can present on the activating group at the position which will be attached to the nucleoside. As

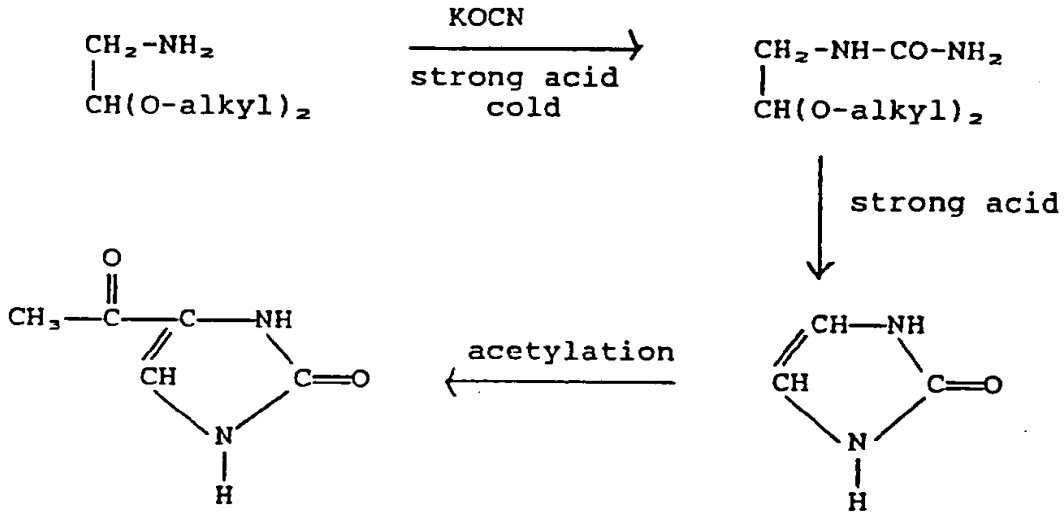
used herein, an activator is an activating group with an attached leaving group.

Moreover, as used herein, activation can occur intermolecularly, intramolecularly or by changing the stereoisomeric configuration of a specific carbon to facilitate attachment of a substituent. Intermolecular activation occurs when an activating group is attached to a oxygen on a precursor for one of the present nucleoside analogs by reacting such a precursor with an activator. When intramolecular activation occurs, a nucleoside oxygen atom which is to be activated is bound to a reactive atom present within the nucleoside. For example, a ribose ring atom can be activated by linkage to a reactive imidazolinone oxygen.

The present compounds are prepared from readily available starting materials. Two generalized synthetic strategies can lead to the present nucleoside analogs. See, for example, Ueda, 1988 in Chemistry of Nucleosides and Nucleotides, Townsend, ed. Vol. 1, pp 1-112. A first, "total synthesis", strategy involves condensing the present 4-acetylimidazolin-2-one base with the desired ribose sugar derivative. A second strategy involves chemical modification of both the heterocyclic base and the sugar moiety of an available nucleoside derivative.

In an exemplary procedure for total synthesis of the present analogs, a free 4-acetylimidazolin-2-one base can be prepared by carbamylation of aminoacetaldehyde dialkylacetal wherein e.g., the alkyl can be ethyl, using a salt of HOCN, such as KOCN in the presence of strong acid (e.g., 5N HCl) at low temperature (e.g., about -40°C). Such a reaction forms

NH_2-CO-NH-CH_2-CH(O-alkyl)$_2$ which can be cyclized to an imidazolinone ring by reaction with acid (e.g., H_2SO_4). An acetyl group can be added to the 4-position of the imidazolinone ring by known procedures, e.g. by using an acetylating agent such as CH_3COCl in the presence of a Lewis acid (e.g. $AlCl_3$). Such a reaction scheme is depicted below.



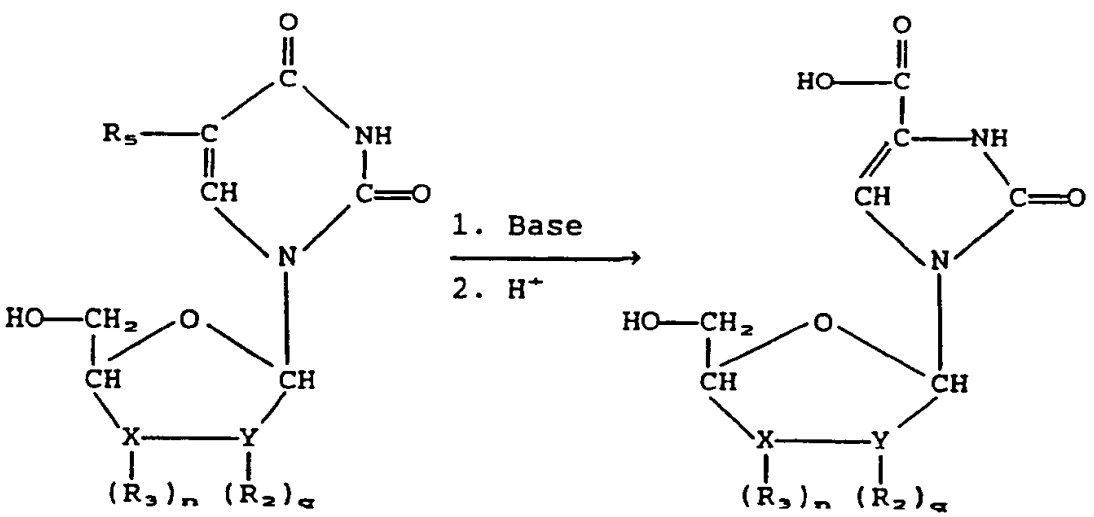
For the total synthesis of a nucleoside analog, the 4-acetylimidazolin-2-one base can be silylated (e.g., with trimethylsilyl, t-butyldimethylsilyl, t-butyldiphenylsilylchloride, trimethyl-t-butyldimethyl-t-butyldiphenylsilylchloride or the like) followed by reaction with the appropriate 1-halo (e.g. chloro or bromo) or 1-acetyl ribose derivative in the absence or presence of a catalyst (e.g. $SnCl_2$, $TiCl_4$ and the like). Such procedures are provided in Coe et al. (1984 Nucleic Acid Res. 12: 6827).

For the second approach, the present imidazolinone nucleoside analogs can be conveniently prepared from commercially available reagents, e.g.,

uridine or, preferably, derivatives of uridine. Particularly useful derivatives of uridine have a free 5'-OH (R_1 is hydroxy) on the ribose and another substituent at the 5-position of the pyrimidine ring, e.g., a 5-halo, 5-hydroxy and the like.

In one exemplary procedure, a particularly useful and readily available derivative of uridine which can be utilized to prepare the present compounds, in particular imidine and its derivatives, is the commercially available 5-bromo-2'-deoxyuridine. After formation of the 4-acetylimidazolinone ring by ring contraction the 2'-deoxyribose ring of this starting material can be modified as described hereinbelow. 5-bromo-2'-deoxyuridine, can be prepared by techniques known to the skilled artisan, e.g., by dissolving a small molar excess of bromine in an aqueous solution of uridine followed by neutralization and deionization.

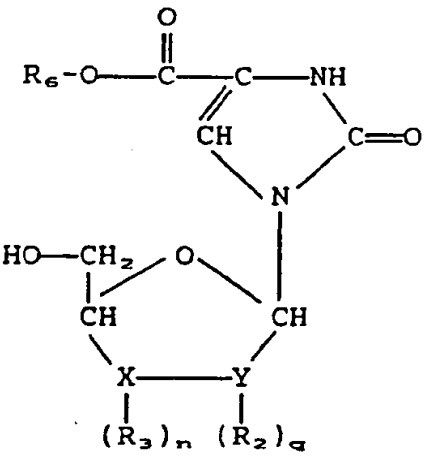
The following reaction schemes illustrate various procedures for synthesizing the nucleoside analogs of the present invention. A uridine derivative (VIII) having a modified ribose ring and a substituent at the 5-position of the pyrimidine ring is reacted with base to promote ring contraction and formation of a imidazolinone intermediate (IX), as depicted below.



VIII IX

The R_5 group is halo or hydroxy and X, Y, R_2, R_3, n and q are as defined hereinabove.

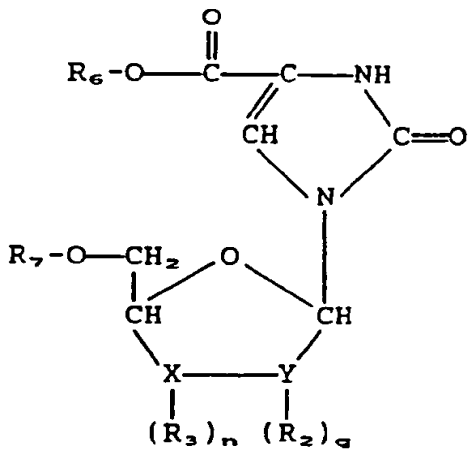
After formation, the carboxylate group at the 4-position of imidazolinone of IX can be protected by addition of a protecting group, e.g., by esterification. Esterification can be performed by known procedures as described in Greene, e.g., by addition of an alcohol such as methanol, ethanol or the like in the presence of acid. This procedure permits higher yields of sugar protected IX after hydrolysis of the ester. The 4-carboxylate-protected derivative of IX has the following structure (X).



X

The R_6 group is a protecting group and X, Y, R_2, R_3, n and q are as defined hereinabove.

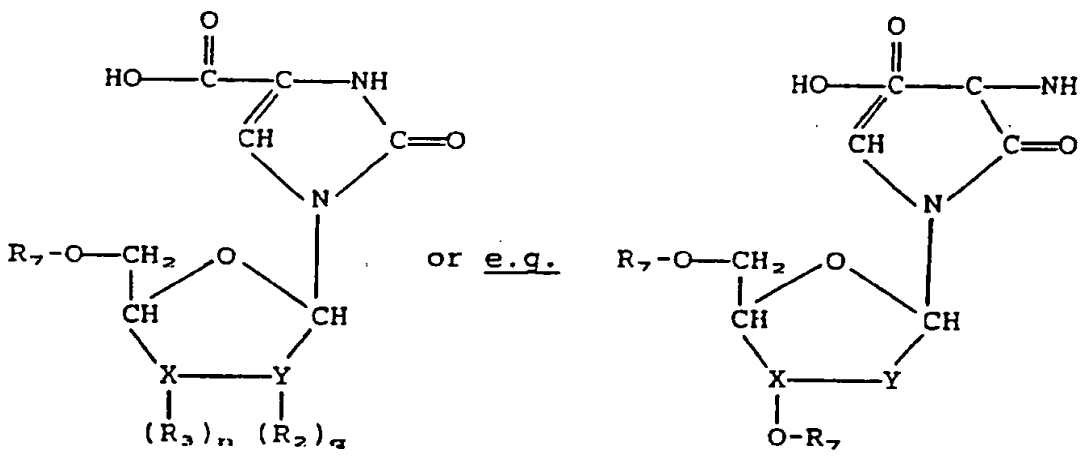
Any free hydroxy groups on the carboxylate protected compound X can be protected by known procedures, e.g., by formation of an alkyl ether, cyclic ether, acetal, ketal, ester and the like. A preferred procedure for protection of such free hydroxy groups is silylation, e.g., using t-butyldimethylsilyl chloride (TBDMSCl) in an anhydrous solvent. Such procedures protect hydroxy groups during conversion of the 4-carboxylate to a 4-acetyl group. One example of a hydroxy protected derivative of compound X, wherein R_2 and R_3 are not hydroxy groups, is depicted below.



XI

R_7 and R_6 are separate protecting groups and X, Y, R_2, R_3, n and q are as defined hereinabove. When R_2 or R_3 is a hydroxy, compound XI will have a R_7-O- substituent in place of the R_2 or R_3 hydroxy.

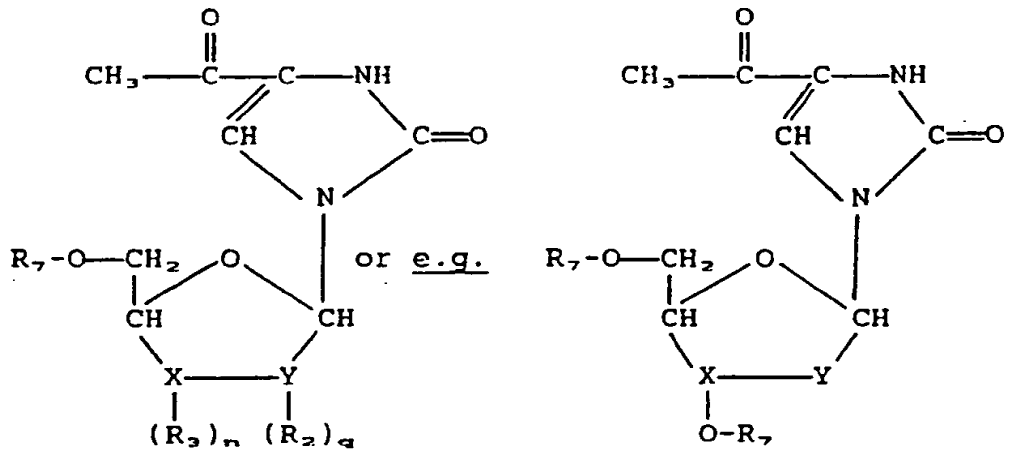
To replace the carboxylate group with an acetyl group the carboxylate protecting group (R_6) is first removed. For example if the carboxylate protecting group is an alkyl, compound XI can be treated with base in an aqueous lower alkanol solvent (e.g., aqueous alcohol). Removal of this protecting group yields compound XII.



XII

R_7, X, Y, R_2, R_3, n and q are as defined hereinabove.

Conversion of the -COOH group of compound XII to $-CO-CH_3$ using methyllithium provides the 4-acetyl imidazolinone nucleoside (XIII). The reaction requires protection of the NH group, preferably by acetylation. Such acetylation can be formed by reacting XII with an acetylating agent, e.g., acetic anhydride, acetyl halide and the like, in nonaqueous solvent.



XIII

R_7, X, Y, R_2, R_3, n and q are as defined hereinabove.

The R_7 hydroxy protecting groups can be removed by known procedures, e.g., when R_7 is silyl, compound XIII can be treated with acid in aqueous solution or with fluoride (e.g., Bu_4NF) to yield a compound of formula I as described hereinabove.

As is recognized by the skilled artisan, alternative procedures can be used for making the present compounds which are adaptions of the procedures described herein or which can include known and commonly available procedures. The procedures provided herein are intended to be illustrative and are not exhaustive; therefore the procedures illustrated herein should not be viewed as limiting the invention in any way.

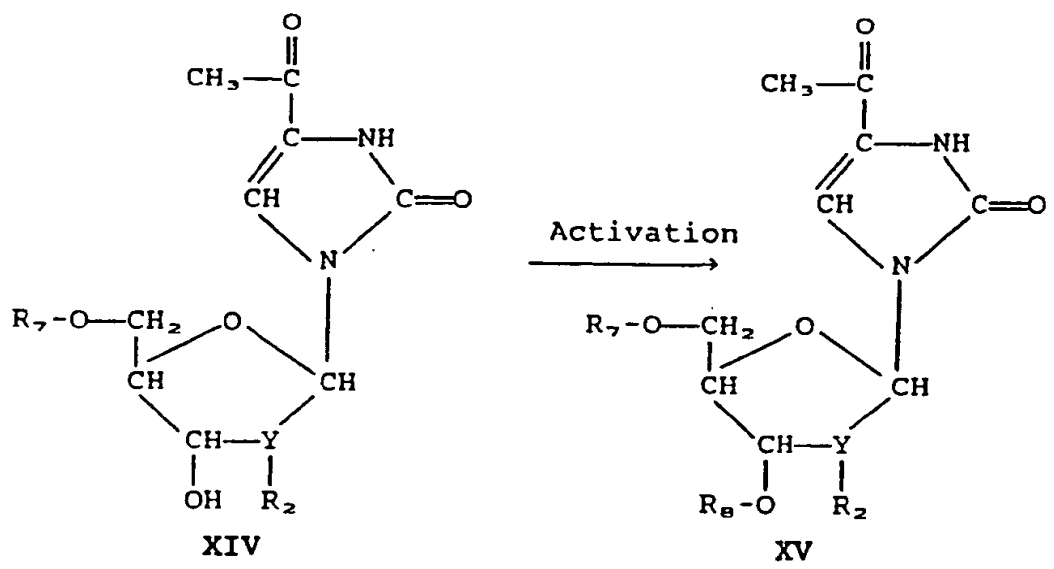
As an alternative to starting with the desired X, Y, R_2 and R_3 groups in place and forming the 4-acetylimidazolinone ring, the desired X, Y, R_2 and R_3 substituents can be added to the ribose ring after synthesis of the 4-acetylimidazolinone ring by art-recognized procedures.

Replacement of a ribose hydroxy with a R_2 or R_3 substituent can require activation of a 2' or 3' hydroxy oxygen. As described above, such activation can occur intermolecularly, intramolecularly or by changing the stereoisomeric orientation of the hydroxy oxygen.

For example, a 3'-OH or a 2'-OH can be activated and replaced by the respective R_3 or R_2 substituent, using a 5'-protected 4-acetylimidazolinone nucleoside (XIV) as a starting reagent, where R_7 is a primary hydroxyl protecting group, preferably trityl, monomethoxytrityl and the like. The XIV starting material can have a 3'-OH if a R_3 group is to replace such a 3'-OH, or the XIV starting material can have a 2'-OH which is replaced with an R_2 group. For example, starting material XIV having a 3'-OH can be activated via XV to form the anhydronucleoside XVI.

Synthetic procedures for replacing a 3'-OH with a R_3 group are illustrated below, but these procedures can readily be adapted by the skilled artisan for replacing a 2'-OH with an R_2 group.

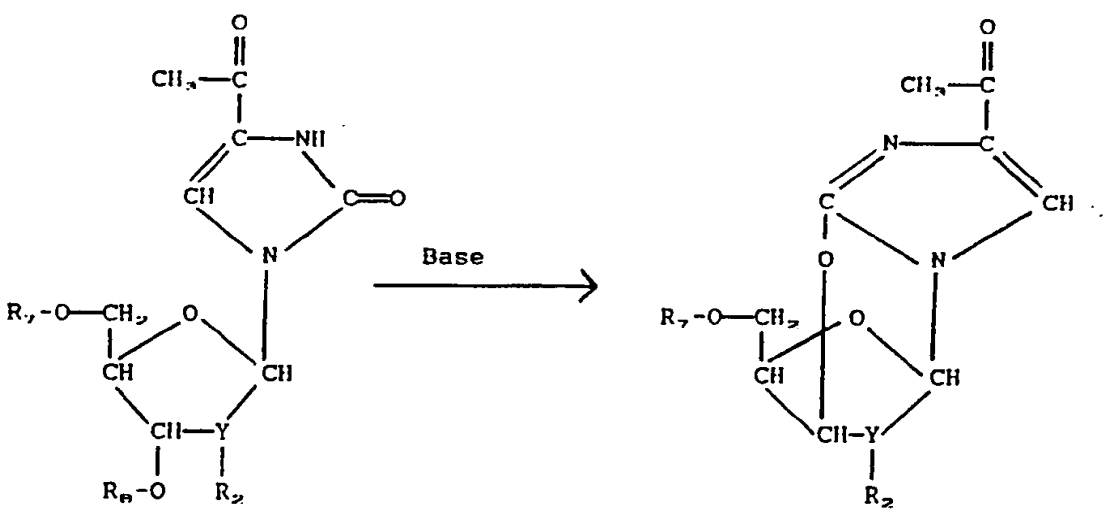
Intermolecular activation of XIV can provide intermediate XV.



XIV XV

R_8 is an activating group and R_7, Y and R_2 are as defined hereinabove.

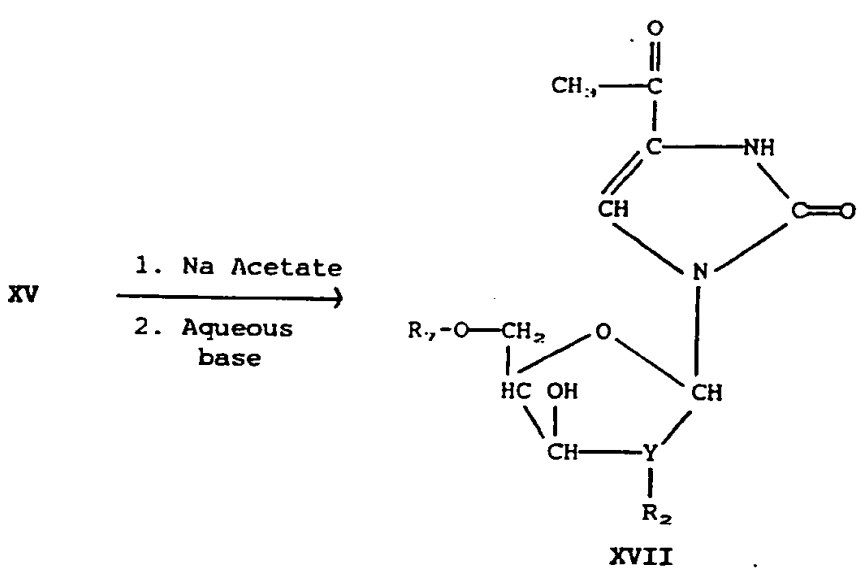
Intramolecular activation requires formation of a bond between the atom to be activated and another atom in the nucleoside, e.g., to intramolecularly activate a ribose oxygen, a covalent linkage can be formed between a 3'-position and the oxygen atom of the 2-carbonyl in the imidazolinone ring. To form such a linkage the stereoisomeric configuration of the 3'-OH must be reversed from the ribo-configuration to the xylo-configuration. For example, a 5'-protected 4-acetyl imidazolinone nucleoside **XV** having an activating group (i.e. R_8 wherein R_8 is preferably mesyl or the like) can be rearranged to form an intramolecularly activated intermediate (**XVI**) by reaction with base, e.g., NaOH , triethylamine and the like.



XV XVI

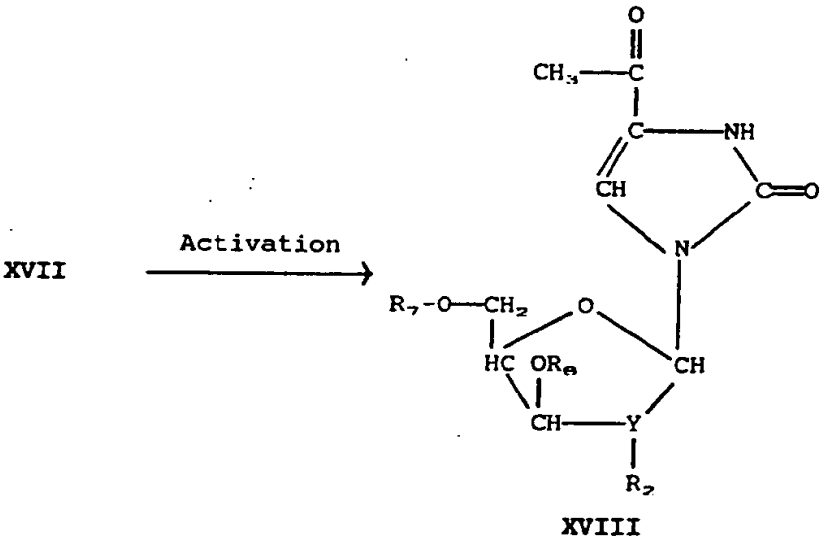
R_8 is an activating group and R_7, Y and R_2 are as defined hereinabove.

Activation can also occur by changing the stereoisomeric configuration of the atom to be activated. For example, a ribose oxygen can be activated for later removal by forming a 3'-OH with the xylo-configuration. In one exemplary procedure, the activated intermediate XV can be reacted with sodium acetate, followed by mild base-catalyzed hydrolysis to produce such a xylose derivative (XVII).



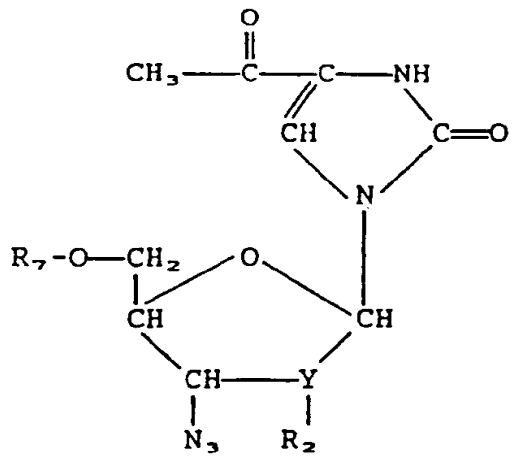
R_7, Y and R_2 are as defined hereinabove.

The xylose 3'-OH of **XVII** can be activated by procedures similar to those described above for the ribose 3'-OH, to produce **XVIII**. Preferred activating groups are mesyl, trifyl and the like.



R_8, R_7, Y and R_2 are as defined hereinabove. Any of the foregoing activated intermediates can be used to replace a 3'-OH present on the selected starting compound with a desired R_3 substituent.

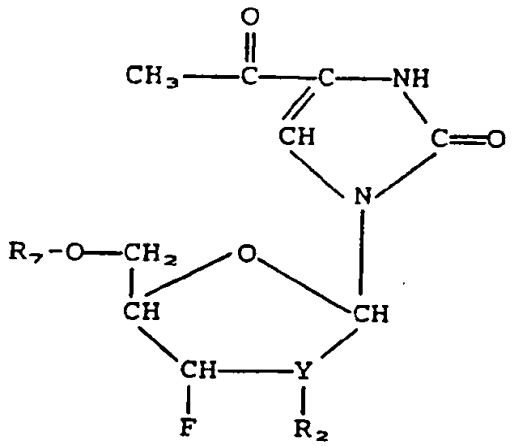
An azido group can be placed on the 3'-position of the ribose ring of the present 4-acetylimidazolinone compounds by known procedures, e.g., as described in Chu et al. (1989 J. Med. Chem. 32: 612-617). Alternatively, the foregoing activated intermediates can be utilized to synthesize a 3'-azido-4-imidazolinone compound (III) of the present invention. For example, an azido group can be placed on any of the 3'-activated intermediates (XV, XVI and XVIII) by heating one these intermediates with sodium azide or lithium azide in a non-aqueous solvent, e.g., dimethyl formamide. A reaction temperature of about 75°C to 150°C can be used. However, compounds XV and XVI may require a somewhat higher reaction temperature than XVIII. A preferred reaction temperature of about 130°C can be used for intermediates XV and XVI, while a reaction temperature of about 100°C or lower temperature is preferred for intermediate XVIII. Such reactions yield a 5'-protected (3-azido-2-deoxy-ß-D-ribofuranosyl)-4-acetylimidazoline-2-one (XIX).



XIX

R_7, Y, R_2 are as defined hereinabove. The 5'-R_7 protecting group can be removed by procedures described hereinabove, e.g. a preferred 5'-trityl protecting group is removed by treatment of XIX with 80% acetic acid.

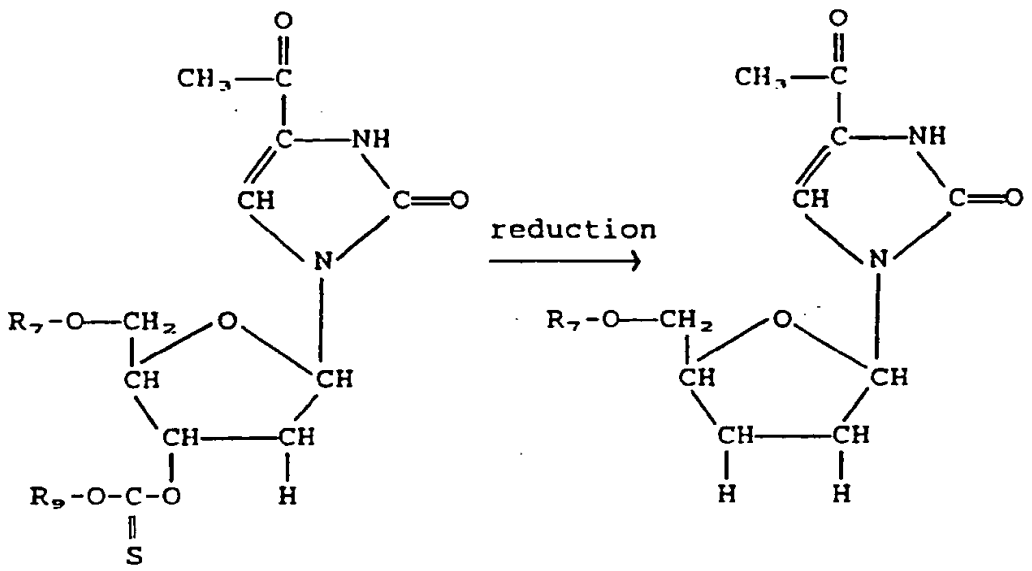
A fluoro group can be placed on the 3'-position of the ribose ring by known procedures, e.g Herdewijn et al. (1987 J. Med. Chem. 30: 1270-1278) and Herdewijn et al. (1989 Nucleoside Nucleotides 8: 65-96). Similarly such a 3'-fluoro compound of the present invention (e.g. compound IV) can be made by reacting either of the 3'-activated XVI or the xylose XVII with a fluorinating agent, e.g., HF (or KHF_2) or diethylamino-sulfur trifluoride (DAST), respectively. Such reactions provide compound XX.



XX

R_7, Y, R_2 are as defined hereinabove. The 5'-R_7 protecting group can then be removed by procedures described hereinabove, e.g. a preferred 5'-trityl protecting group is removed by treatment of XX with 80% acetic acid.

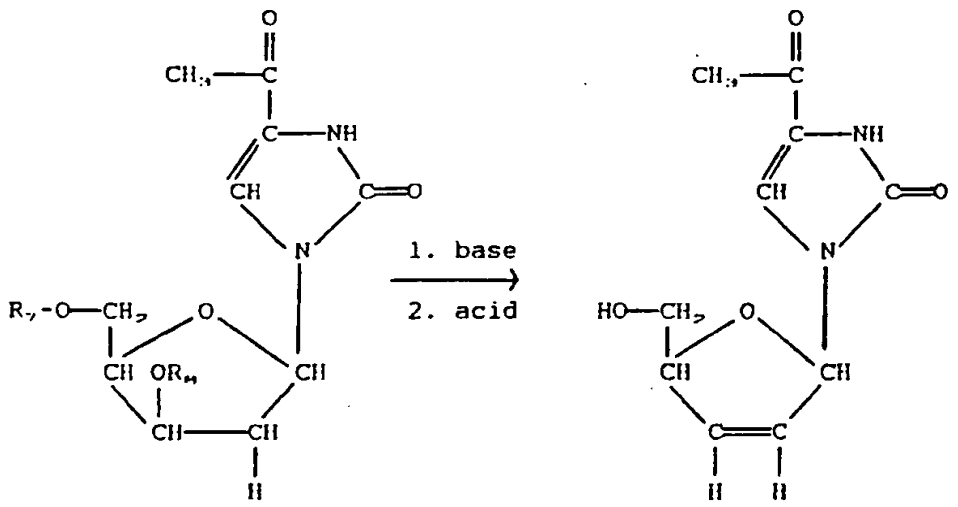
A 2',3'-dideoxyribose analog of the present invention can be made e.g., by the method of Prisbe et al. (1985 Synthetic Commun. 15: 401-409) or Robins et al. (1983 J. Am. Chem. Soc. 105: 4059-4065). For example, a thionocarbonate compound (XXI, wherein R_8 is methyl, phenyl and the like) can be formed by reaction of XIV with phenyl chlorothionocarbonate or methyl chlorothionocarbonate. Thionocarbonate compound XXI can be reduced, e.g. by tin hydride in the presence of azobisisobutyronitrile (AIBN). The compound formed from this reaction is a 5'-protected 2',3'-dideoxyribo-furanosyl-4-acetylimidazolinone (XXII).



XXI XXII

The 5'-R_7 protecting group can then be removed by procedures described hereinabove, e.g. a preferred 5'-trityl protecting group is removed by treatment of XXII with 80% acetic acid or HCl in chloroform.

A 2',3'-unsaturation can also be created within a ribose ring present on the subject compounds, e.g., as in Horwitz et al. (1966 J. Org. Chem. 29: 205). A 2'deoxy-derivative of intermediate XVII can be used for synthesizing such a 2',3'-unsaturated compound. For example, intermediate XVIII can be reacted with a strong base, e.g., tetrabutylammonium fluoride (TBAF), potassium tertiary butoxide (t-Bu-OK) and the like, followed by acid catalyzed removal of the R_7 protecting group to provide a 2',3'-unsaturated compound (VI) of the present invention, as shown below.



XVIII VI

A dioxolane-4-acetylimidazolinone compound (VII) of the present invention (wherein X is O) can be made by procedures available to the skilled artisan, e.g., by the method of (Choi et al. 1991 J. Amer. Chem. Soc. 113: 9377-9379).

A modified mono-, di- or tri-phosphate can be placed on the nucleoside analogs by any method available to the skilled artisan. For example, Uhlmann et al. (1990, Chemical Reviews 90: 543-584) provide references and outline procedures for making nucleotides with modified phosphates. The preferred 5'-monophosphates are conveniently prepared by the method of Yoshikawa (1969 Bull. Chem. Soc. (Japan) 42: 3505). The corresponding preferred 5'-triphosphates can be obtained by pyrophosphorylation of the 5'-monophosphate (e.g. as in Kovacs et al. 1988 Tetrahedron Lett. 29: 4525). The preferred 5'-phosphonates and the corresponding 5'-

triphosphates can be prepared as described by Freeman et al. (1992 J. Med. Chem. 35: 3192-3196).

The present compounds which contain one or more phosphates can form salts with cations. All such cationic salts are contemplated by the invention, but preferred salts are formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, barium, ammonium, monoethanolammonium, tri-(cyclohexylammonium) and similar cations well known in this art.

In another embodiment the present invention provides a pharmaceutical composition containing a pharmaceutically effective amount of at least one of the present compounds.

As used herein such a pharmaceutically effective amount is an anti-viral effective amount, a reverse transcriptase-inhibiting amount, a retrovirus replication-inhibiting amount, a hepatitis B replication-inhibiting amount or a human immuno-deficiency virus-inhibiting amount. According to the present invention the pharmaceutically effective amount is chosen as one that does not substantially inhibit mammalian DNA replication mediated by cellular DNA polymerases, including nuclear and mitochondrial DNA polymerases. In particular the present compounds generally inhibit reverse transcriptase at about 500-fold lower concentrations than required for inhibition of mammalian DNA polymerases involved in cell replication.

The compounds of the present invention are generally administered to birds and mammals, including but not limited to humans. Generally the mg/kg/day

dosage required for humans is less than that required for small warm-blooded animals, e.g. mice.

A pharmaceutically effective amount of the present compounds is about 0.001 mg/kg/day to about 500 mg/kg/day as needed to attain beneficial therapeutic effects. In a preferred embodiment such a pharmaceutically effective amount of the present compounds is about 0.01 mg/kg/day to about 300 mg/kg/day. For example, about 1 mg to about 500 mg of the present compounds can be administered approximately every 4-12 hr. Specific dosage amounts can be readily determined by one of ordinary skill in the art taking into account factors which generally tend to modify drug action, e.g. age, weight, sex, diet, disease state, times and methods of administration, and the like.

A dosage unit can include a single compound of the present invention or a mixture of the present compounds; a dosage unit can further include other therapeutic agents beneficial for the treatment of diseases caused by retroviruses or hepatitis B viruses. Such combinations of the present compounds with other therapeutic agents can be administered either sequentially or simultaneously.

The compounds of the present invention can be administered to an animal in a variety of forms adapted to the chosen route of administration, e.g., oral, topical, intradermal, intravenous, intramuscular, intraperitoneal or subcutaneous routes. The subject compounds can also be administered parenterally by osmotic pump to permit continuous infusion of the active compound, for example, as described in Rataiczak et al. (1992 Proc. Natl. Acad. Sci. USA 89: 11823-11827). Such

osmotic pumps are commercially available, e.g., from Alzet, Inc (Palo Alto, CA).

For oral administration the present nucleoside analogs can be suitably protected, e.g., by enclosure in hard or soft shell gelatin capsules. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, incorporated directly with the food of the diet and the like. The subject compounds can be incorporated into a cream, solution or suspension for topical administration. The active compounds may be incorporated into liposomes or liposomes modified with polyethylene glycol for parenteral administration. Incorporation of additional substances into the liposome, for example, antibodies reactive against membrane proteins found on specific target cells, can help target the present compounds to specific cell types.

The percentage of such additives and stabilizers can be varied as needed, however the amount of active compound is at least 0.1%. More conveniently the active compound can constitute about 2% to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is varied such that a suitable dosage will be obtained. Compositions according to the present invention are prepared in unit dosage form so that an oral dosage unit form contains an amount ranging from about 0.01 mg to about 1 g of active compound. Preferred dosage ranges from about 0.01 mg to about 500 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: a binder such as gum tragacanth, acacia, corn starch or gelatin; an excipient such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid or the like; a lubricant such as magnesium stearate; a sweetening agent such as sucrose, fructose, lactose or saccharin; or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it can also contain a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir can contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. In addition, the active compound may be incorporated into sustained-release preparations and formulations. Any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed.

The active compound may also be administered parenterally or intraperitoneally. Solutions of the active compound as a free base, acid or pharmacologically acceptable salt can be prepared in water. Such solutions can be mixed with a surfactant such as hydroxypropylcellulose or a dispersing agent such as glycerol, a liquid polyethylene glycol, an oil and a mixture thereof. Under ordinary conditions of storage and use these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and preserved against the contaminating action of microorganisms such as bacteria and fungi. Such pharmaceutical forms for injection must be fluid to the extent that easy syringability exists. Preferably the pharmaceutical composition is stable under the conditions of manufacture and storage.

A pharmaceutical carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, polyethylene glycol and the like), vegetable oil and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other

ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solutions thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The following examples further illustrate the invention and are not intended to limit the invention.

EXAMPLE 1

SYNTHESIS OF 1-(β-D-2-DEOXYRIBOFURANOSYL)-4-ACETYLIMIDAZOLIN-2-ONE (dImd)

Materials and Methods

Melting points were determined on a MEL-TEMP apparatus. ^{1}H-NMR spectra were recorded at 300 MHz on a Varian Gemini spectrometer. Thin layer chromatography (TLC) was performed on ANALTECH Hard Layer GHLF UNIPLATE and spots were examined under UV light. Elemental analysis were carried out by Atlantic Microlab, Norcross, Georgia.

Methyl 1-(2-Deoxy-β-D-ribofuranosyl)imidazolin-2-one-4-carboxylate (2)

5-Bromo-2'-deoxyuridine (Aldrich) 1 (3.07 g, 10 mmol) was dissolved in a solution of sodium bicarbonate (2.52 g, 30 mmol) in 200 mL of water. The alkaline solution was refluxed for 20 hours under N_2, until no starting material remained, as shown by TLC. The reaction mixture was passed through a column of ion exchange resin (Dowex 50W X8, 100-200 mesh, H^+ form) to convert the sodium salt of the product into the free acid. The solution was concentrated under reduced pressure. The black residue obtained was dissolved in 100 mL of MeOH, to which a solution of diazomethane in ether was added portionwise at 4°C with thorough stirring. The reaction was monitored by TLC ($CHCl_3$/MeOH/HOAc = 6:2:0.5, v/v), until all carboxylic acid was consumed. The solvent was removed under diminished pressure and the residue was absorbed on 10 g of silica gel. The product was purified using column

chromatography (CH_2Cl_2/MeOH = 20:1, v/v) to give a foamy substance which crystallized in MeOH to yield 1.59 g of 2 as a white powder (61%). M.P. 157-160°C; ^{1}H-NMR (DMSO-d_6) δ 1.84-2.08 (m, 1H, 2'-H), 2.20-2.33 (m, 1H, 2'-H), 3.51 (m, 2H, 5'-H), 3.73 (m, 4H, $COOCH_3$, 3'-H), 4.20 (m, 1H, 4'-H), 4.91 (m, 1H, 5'-OH), 5.21 (m, 1H, 3'-OH), 5.84 (m, 1H, 1'-H), 7.59 (s, 1H, 5-H), 10.93 (br, 1H, NH). Anal. calc'd for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.85. Found: C, 46.51; H, 5.48; N, 10.77.

Methyl 1-(2-Deoxy-3,5-di-O-t-butyldimethylsilyl-β-D-ribofuranosyl)imidazolin-2-one-4-carboxylate (3)

To the solution of compound 2 (3.78 g, 15 mmol) and imidazole (4.5 g, 66 mmol) in 30 mL of anhydrous DMF, t-butyldimethylsilyl chloride (TBDMSCl) (4.97 g, 33 mmol) was added. The reaction mixture was stirred for 24 hours at room temperature. After removal of the solvent under reduced pressure, the residue was dissolved in 30 mL of $CHCl_3$, washed 3X with water, and dried over anhydrous Na_2SO_4. The product was recrystallized from MeOH/H_2O (10:2) to yield 7.2 g of 3 as a white powder (95%). ^{1}H-NMR ($CDCl_3$) δ 0.10 (m, 12H, $Si(CH_3)_2$), 0.93 (m, 18H, $SiC(CH_3)_3$), 2.12-2.19 (m, 2H, 2'-H), 3.77 (m, 2H, 5'-H), 3.82 (m, 3H, $COOCH_3$), 3.91 (m, 1H, 3'-H), 4.54 (m, 1H, 4'-H), 6.09 (m, 1H, 1'-H), 7.33 (s, 1H, 5-H), 8.46 (br, 1H, NH). Anal, calc'd for $C_{22}H_{42}N_2O_6Si_2 \cdot H_2O$: C, 52.34; H, 8.79; N, 5.55. Found: C, 52.67; H, 8.53; N, 5.56.

1-(2-Deoxy-3,5-di-O-t-butyldimethyl-β-D-ribofuranosyl)imidazolin-2-one-4-carboxylic Acid (4)

To the solution of compound 3 (2.43 g, 5 mmol) in 20 mL of dioxane, 5 mL of aqueous 1N NaOH was added.

The mixture was refluxed until most of the starting material was consumed (about 16 (hours). The solvent was removed under reduced pr ssure and the residue was dissolved in 80 mL of MeOH. The sodium salt of the product was converted to the free carboxylic acid by contact with an ion exchange resin (Dowex 50W X8, H^+ form, 100-200 mesh). The mixture was filtered, and the resin was washed thoroughly with MeOH. The filtrate and washings were combined and concentrated under diminished pressure below 30°C to yield 1.96 g white crystals of 4 (82.9%). An analytical sample was recrystallized from MeOH/H_2O. M.P. 198-202°C (dec.); ^{1}H-NMR($CDCl_3$) δ 0.05 (m, 12H, $Si(CH_3)_2$), 0.95 (m, 18H, $Si(CH_3)_3$), 2.25 (m, 2H, 2'-H), 3.76 (m, 2H, 5'-H), 3.93 (m, 1H, 3'-H), 4.50 (m, 1H, 4'-H), 6.09 (m, 1H, 1'-H), 7.28 (s, 1H, 5-H), 10.65 (s, 1H, NH), 14.05 (br, 1H, COOH). Anal. calc'd for $C_{21}H_{40}N_2O_6Si_2$: C, 53.35; H, 8.53; N, 5.93. Found: C, 53.06; H, 8.32; N, 5.96.

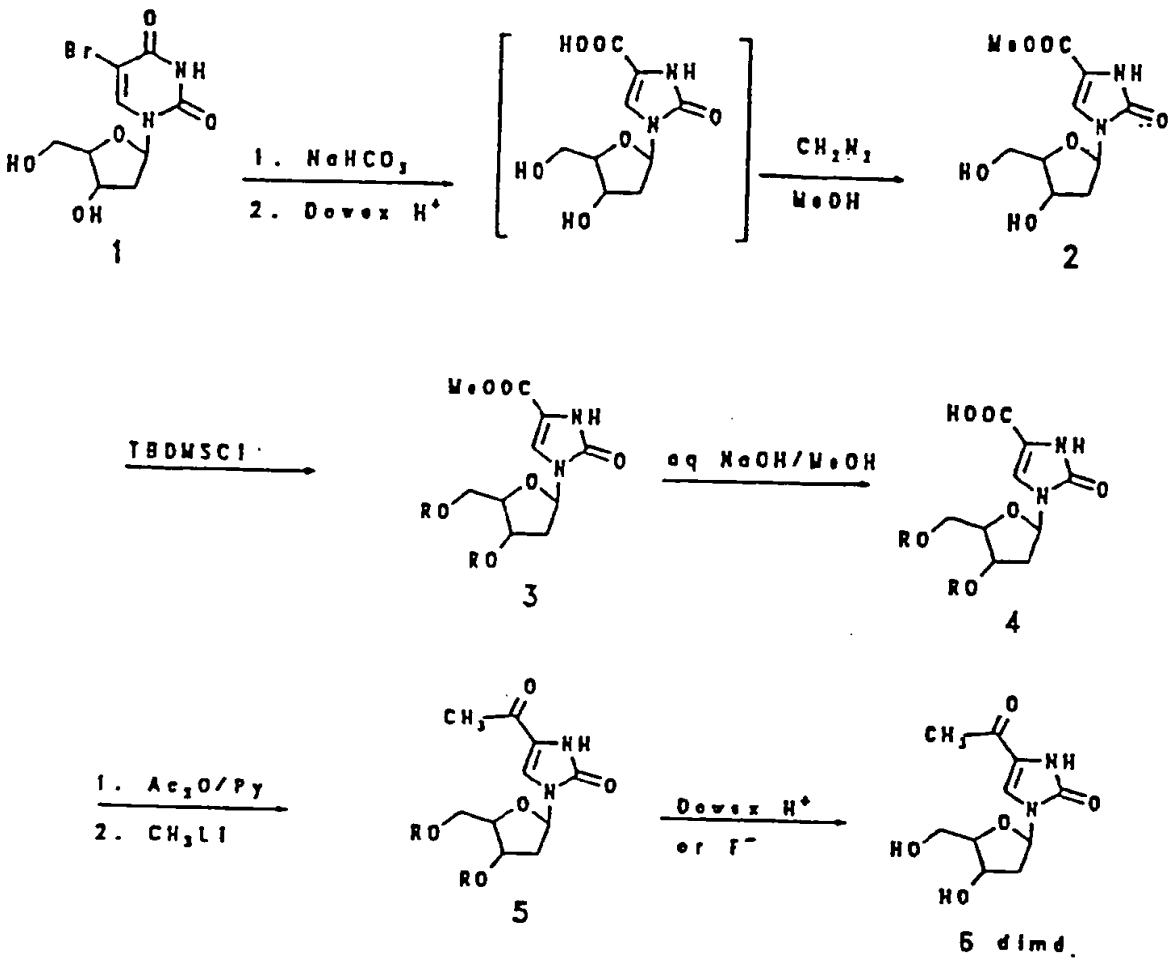
1-(2-Deoxy-3,5-di-O-t-butyldimethylsilyl-β-D-ribofuranosyl)-4-acetylimidazolin-2-one (5)

To compound 4 (1.42 g, 3 mmol) dissolved in 15 mL pyridine, 2 mL acetic anhydride was added. The reaction mixture was stirred at room temperature overnight. The excess reagent and pyridine were removed under reduced pressure at a temperature below 40°C to yield a light brown thick syrup. Methyllithium in 21 mL ether was added to 25 mL of toluene at 0-4°C. The light brown thick syrup was dissolved in 17 mL toluene and added dropwise to the methyllithium solution, while stirring and maintaining the temperature at 40-45°C. Such dropwise addition was continued for 1.5 h at the

same temperature. The reaction mixture was poured into 200 mL of ice water and neutralized with 2N HCl solution. The organic layer was separated and the water layer was extracted with ether. The organic layers were combined, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by chromatography on a silica gel column to yield 510 mg of compound 5 (36%) as a pure syrup. ^{1}H-NMR ($CDCl_3$) δ 0.08 (m, 12H, $Si(CH_3)_2$), 0.9 (m, 18H, $SiC(CH_3)_3$), 2.15 (m, 2H, 2'-H), 2.26 (s, 3H, $COCH_3$), 3.75 (m, 2H, 5'-H), 3.91 (m, 1H, 3'-H), 4.41 (m, 1H, 4'-H), 6.05 (m, 1H, 1'-H), 7.24 (s, 1H, 5-H), 7.91 (br, 1H, NH). Anal. calc'd for $C_{22}H_{42}N_2O_5Si_2$: C, 56.13; H, 8.99; N, 5.95. Found: C, 55.97; H, 9.02; N, 5.85.

1-(2-Deoxy-β-D-ribofuranosyl)-4-acetylimidazolin-2-one (dImd, 6)

To the solution of compound 5 (1.25 g, 2.66 mmol) in a mixture of MeOH/H_2O (5:1), 15 g of ion exchange resin (Dowex 50W X8, H^+ form) was added. The reaction mixture was stirred at room temperature overnight followed by filtration. The filtrate was concentrated to dryness and the residue was purified using silica gel column chromatography to yield 553 mg of compound 6 as a white powder (86%). M.P. 179-182°C; ^{1}H-NMR(DMSO-d_6) δ 2.08 (m, 1H, 2'-H), 2.24 (s, 3H, $COCH_3$), 2.29 (m, 1H, 2'-H), 3.50 (m, 2H, 5'-H), 3.73 (m, 1H, 3'-H), 4.26 (m, 1H, 4'-H), 4.90 (t, 1H, 5'-OH), 5.21 (d, 1H, 3'-OH), 5.84 (t, 1H, 1'-H), 7.84 (s, 1H, 5-H), 10.77 (s, 1H, NH). Anal. calc'd for $C_{10}H_{14}N_2O_5$: C, 49.58; H, 5.83; N, 11.57. Found: C, 49.66; H, 5.84; N, 11.56.

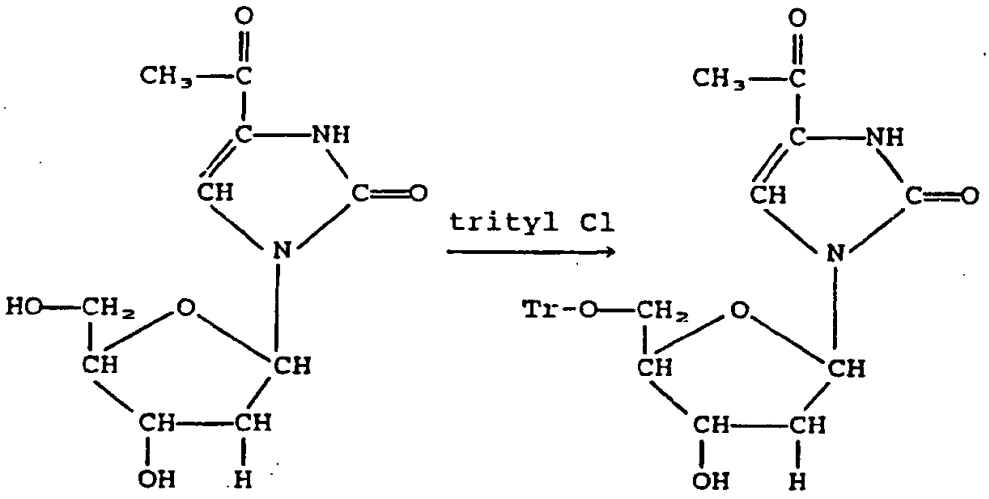


EXAMPLE 2

SYNTHESIS OF 1-(ß-D-2,3-DIDEOXYRIBOFURANOSYL)-4-ACETYLIMIDAZOLIN-2-ONE (ddImd)

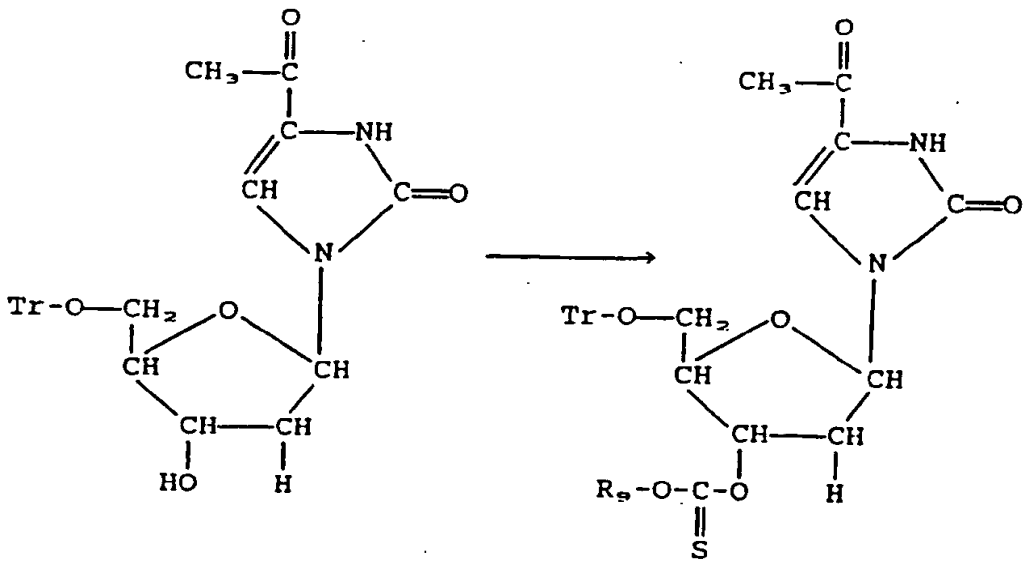
A 2',3'-dideoxy analog of imidine was made by modification of the method of Robins et al. (1983 J. Am. Chem. Soc. 105: 4059-4065).

1-(2-Deoxy-ß-D-ribofuranosyl)-4-acetylimidazolin-2-one (dImd) was synthesized as described in Example 1. This compound had a 2'-deoxy, a 3'-OH and a 5'-OH. dImd was treated with trityl chloride in pyridine to place a trityl (Tr) protecting group on the 5'-OH and thereby generate compound 7.



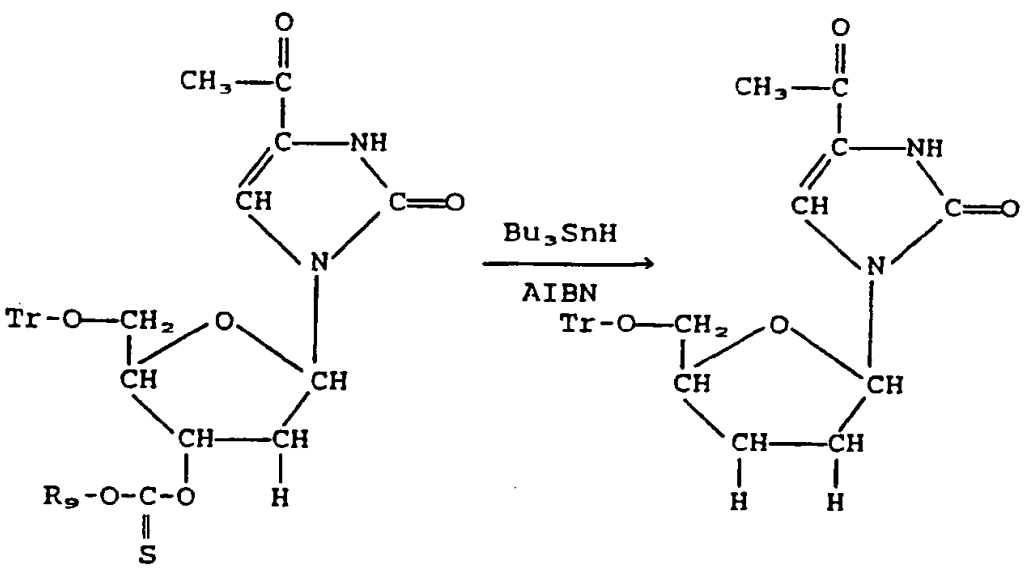
dImd (6) 7

Compound 7 was treated with phenyl chlorothionocarbonate in acetonitrile at 70° to 75°C to generate the thionocarbonate intermediate 8.



7 8

The thionocarbonate (8) was then reduced with tri-n-butyltinhydride (Bu_3SnH) in the presence of azobisisobutyronitrile (AIBN) at 75°C to 80°C, using toluene as solvent. This reaction produced a 5'-trityl-1-(2,3-dideoxy-β-D-ribofuranosyl)-4-acetylimidazolin-2-one compound 9.



8 9

The 5'-trityl protecting group was removed by treatment of compound 9 with 80% acetic acid in chloroform to provide 1-(2,3-dideoxy-β-D-ribofuranosyl)-4-acetylimidazolin-2-one, i.e. compound V.

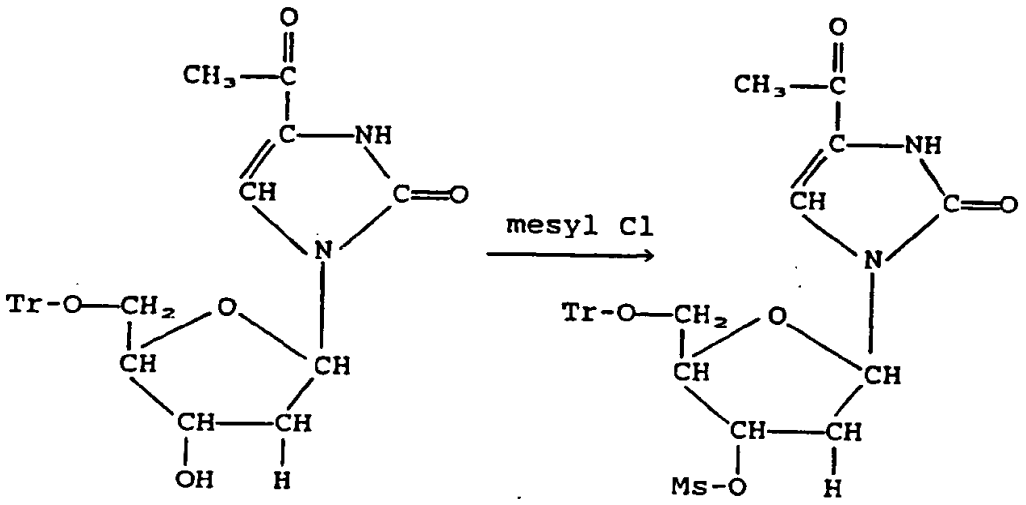
EXAMPLE 3

SYNTHESIS OF A 1-(ß-D-2,3-DIDEOXY-2,3-DIDEHYDRORIBOFURANOSYL)-4-ACETYLIMIDAZOLIN-2-ONE

A 2',3'-dideoxy-2',3'-didehydro analog of imidine was made by modification of the method of Horwitz et al. (1966 J. Org. Chem. 29: 205).

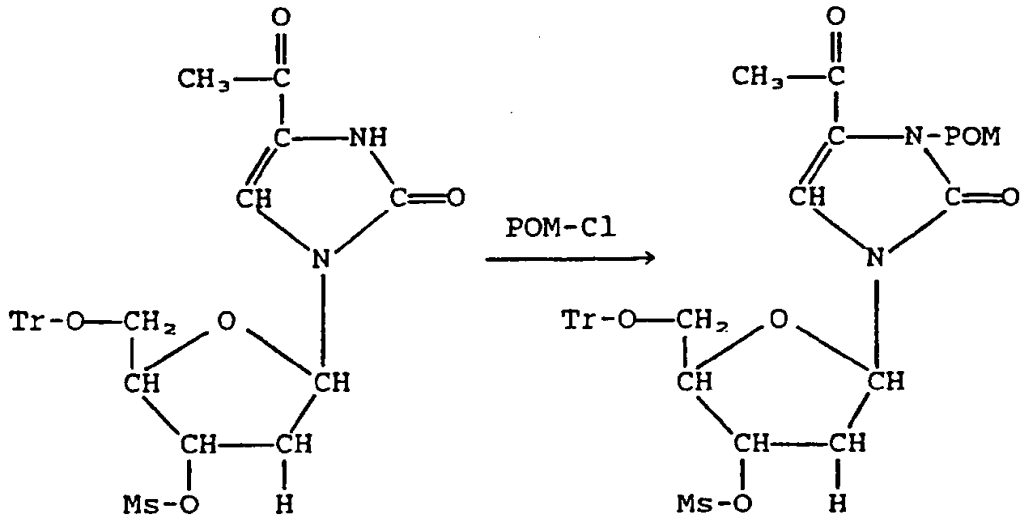
1-(2-Deoxy-ß-D-ribofuranosyl)-4-acetylimidazolin-2-one (dImd) was synthesized as described in Example 1. This compound had a 2'-deoxy, a 3'-OH and a 5'-OH. dImd was treated with trityl chloride in pyridine to place a trityl (Tr) protecting group on the 5'-OH and thereby generate compound 7, as described in Example 2.

Compound 7 was treated with mesyl chloride at room temperature using dimethylformamide as solvent to place a mesyl (Ms) group on the 3'OH. This reaction yielded compound 10, depicted below.



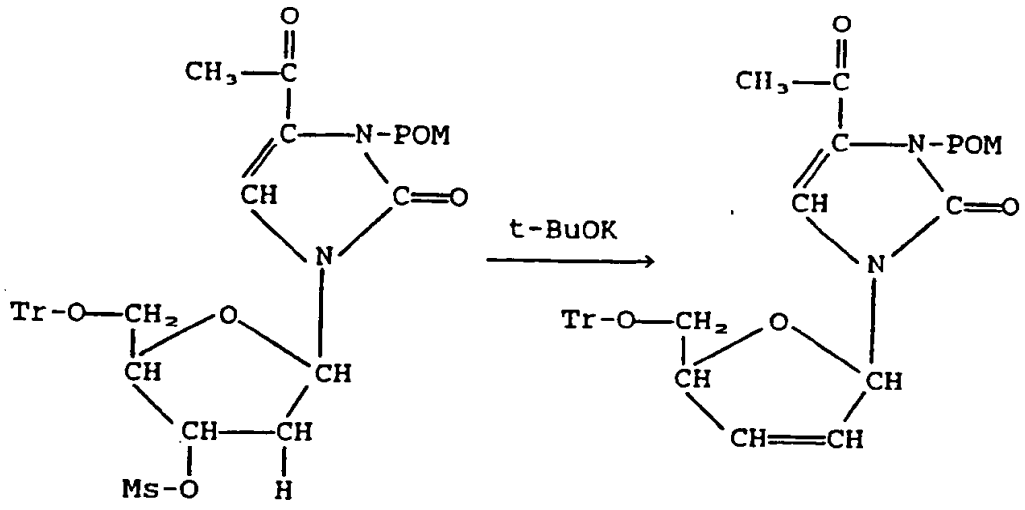
7 10

The ring NH of compound 10 was then protected to prevent cyclization which leads to a compound analogous to XVI. Compound 10 was treated with pivaloyloxy-methyl chloride (POM-Cl) in dimethylformamide and potassium carbonate at room temperature to yield compound 11.



10 11

Compound 11 was then treated with potassium tertiary butoxide (t-BuOK) or sodium acetate to generate the 2',3'-unsaturated compound 12.



11 12

The POM protecting group was removed from compound 12 using concentrated ammonium hydroxide in methanol to generate compound 13. The 5'-trityl protecting group was removed by treatment of compound 13 with 80% acetic acid in chloroform to provide the 2',3'-unsaturated-1-(ß-D-ribofuranosyl)-4-acetylimidazolin-2-one, i.e. compound VI.

EXAMPLE 4

COMPETITIVE INHIBITION OF REVERSE TRANSCRIPTASE WITHOUT INHIBITING CELLULAR DNA POLYMERASE

The amount triphosphate of 1-(2-deoxy-ß-D-ribofuranosyl)-4-acetylimidazolin-2-one (dImdTP) required to inhibit the activity of HIV reverse transcriptase was determined to be about 500-fold less than the amount of dImdTP required to similarly inhibit the activity of human nuclear DNA polymerase α.

Materials and Methods

dImd having a 2'-deoxy, a 3'-OH and a 5'-OH was prepared as described in Example 1. The 5'-triphosphate derivative of dImd was prepared by direct phosphorylation of the 5'-OH using phosphorous-oxychloride ($POCl_3$) to form the 5'-monophosphodichloridate, followed by pyrophosphorylation according to the method of Kovacs et al. (1988 Tetrahedron Lett. 29: 4525).

HIV reverse transcriptase was obtained from the National Institutes of Health AIDS Reagent Repository (catalog no. 1249).

DNA polymerase α was isolated from MOLT-4 human lymphocytes according to the procedure of Ho et al. (1985 Cancer Biochem. Biophys. 8: 85-94).

The template for reverse transcription was poly-rA (Pharmacia) using an oligo dT primer (Pharmacia). Activated calf thymus DNA served as the template for MOLT-4 human lymphocyte DNA polymerase α.

Assay of Reverse Transcriptase

The inhibition of HIV reverse transcriptase (RT) was determined by measuring the incorporation of radioactive ^{3}H-dTTP into a synthetic template-primer poly(rA)$_n$•(dT)$_{12-18}$ in the presence of varying concentrations of the nucleoside analog triphosphate. A control reaction contained no nucleoside analog triphosphate. The concentration of analog required for 50% inhibition of the control activity is referred to as the IC_{50}.

The assay for HIV reverse transcriptase was performed essentially as described by Eriksson et al. (1989 Antimicrobial Agents and Chemotherapy 33: 663-669). The standard reaction mixture contained, in a total volume of 100 µL, 100 mM Tris-HCl buffer (pH 8.0), 50 mM KCl, 2 mM $MgCl_2$, 5 mM dithiothreitol, 9 µg/mL bovine serum albumin, 0.001 O.D. unit (0.06 µg) of poly(rA)$_n$•(dT)$_{12-18}$, 0.13 µM ^{3}H-dTTP (specific activity 47 Ci/mmol) and 10 µL HIV reverse transcriptase (0.16 unit). After incubation for 1 hour at 37°C, the reaction was terminated by addition of 1 mL 10% trichloroacetic acid (TCA) containing 0.1 M Na-pyrophosphate. After standing on ice for 10 min., the resulting precipitate was collected on a glass microfiber filter disk, washed twice with 1 ml 5% TCA twice followed by 0.6 ml ethanol, then dried under infrared light. The radioactivity was measured by placing the filter in a vial containing 7 mL Ecoscint (National Diagnostics) and counted in a Packard (Model 1900 TR) liquid scintillation analyzer.

Assay of MOLT-4 DNA Polymerase α

The inhibition of human nuclear DNA polymerase α from MOLT-4 cells was assayed as described for HIV reverse transcriptase, except that 16 µg activated calf thymus DNA was used as a template-primer and all required nucleotide substrates were provided at 0.1 mM (i.e., dATP, dCTP and dGTP). ^{3}H-dTTP (0.13 µM with specific activity 47 Ci/mmol) was used as described above.

Results

Fig. 4 provides a graph of the percent inhibition of HIV reverse transcriptase and normal human DNA polymerase activities in the presence of various concentrations of 1-(2-Deoxy-ß-D-ribofuranosyl)-4-acetylimidazolin-2-one 5'-triphosphate (dImdTP) analog inhibitor. As illustrated, the concentration of analog inhibitor required for 50% inhibition (IC_{50}) of human immunodeficiency virus reverse transcriptase was found to be 38 nM. In contrast the IC_{50} for normal human DNA polymerase α was determined to be about 500-fold higher, i.e., 17 µM.

A mathematical analysis of the graphic data depicted in Fig. 4 is provided in Tables 1A and 1B.

TABLE 1A

Inhibition of HIV
Reverse Transcriptase by dImdTP

Sigmoid curve (log scale)
A=bottom, B=top, C=log(EC50), D='Hill' Slope

Final Results Sum of Squares = 27.39 (df=3)
Goodness-of-fit assessed using actual distances; R squared = 0.933.

Parameter	Value	Approx. SE	%Error (CV)
A	0	(Constant)	----
B	98.8	3.64	3.7%
C	-7.42	0.054	0.7%
D	1.00	0.117	11.7%

The IC_{50} value determined for HIV reverse transcriptase was 3.8×10^{-8} molar, i.e., 38 nM.

TABLE 1B

Inhibition of
Human DNA Polymerase α by dImdTP

Sigmoid curve (log scale)
A=bottom, B=top, C=log(EC50), D='Hill' Slope

Final Results Sum of Squares = 25.25 (df=2)
Goodness-of-fit assessed using actual distances; R squared = 0.994.

Parameter	Value	Approx. SE	%Error (CV)
A	0	(Constant)	----
B	119.	16.33	13.7%
C	-4.77	0.198	4.2%
D	.751	0.128	17.0%

The IC_{50} value determined for MOLT 4 human DNA polymerase α was 1.7 X 10^{-5} molar, i.e., 17 μM. These data indicate that dImdTP is a much more potent (500-fold) inhibitor of HIV reverse transcriptase than of human DNA polymerase. Moreover the observed diminution in reverse transcriptase activity occurred by selective competitive inhibition for reverse transcriptase rather than by chain termination since the dImdTP analog tested had a free 3'-OH.

EXAMPLE 5

INHIBITION OF HIV INFECTION IN CULTURED CELLS WITHOUT CYTOTOXICITY

Materials and Methods

dImd having a 2'-deoxy, a 3'-OH and a 5'-OH was prepared as described in Example 1.

HIV-1 was originally obtained from the culture supernatant of a persistently HIV-infected H9 cell line, H9-HTLV-III_B, described in Popovic et al. (1984 Science 224: 497-500). For the experiments described below, HIV-1 stocks were prepared from the supernatants of HIV-1 infected MOLT-4 human T-lymphocytes. Similarly, HIV-2 stocks were prepared from the supernatants of HIV-2 infected MOLT-4 human T-lymphocytes.

The procedure for testing the effectiveness of the present analogs against HIV was essentially as described in Balzarini et al. (1991 AIDS 5: 21-28) and Balzarini et al. (1988 Biochem. Pharmacol. 37: 2847-2856). MOLT-4 and CEM cells (5 x 10^5 cells ml) were suspended in fresh culture medium and infected with either HIV-1 or HIV-2 at 100% to 50% cell culture infective doses (CCID) per ml cell suspension ($CCID_{50}$ is the dose required for infection of about 50% of the cultured cells). 100 µl infected cell suspension were transferred to microtiter plate wells, mixed with 100 µl of the appropriate dilutions of dImd and further incubated at 37°C. Cells were then pelleted, suspended in fresh RPMI-1640 culture medium containing 13% fetal calf serum (FCS), 11% interleukin-2 (vol/vol), 50 µmol/l ß-mercaptoethanol, 4 mmol/l L-glutamine, 50 units/ml penicillin and 50 µg/ml streptomycin, and infected with

2 x 10^3 HIV virions/cell for 60-90 min at 37°C. After infection, cells were reconstituted in culture medium and seeded in culture tubes at 2 ml per tube in the presence or absence of the test compound. After 5 days, the number of viable cells was determined in a blood-cell-counting chamber by Trypan blue staining for both virus-infected and mock-infected cell cultures. The 50% effective dose (ED_{50}) was defined as the concentration of compound required to reduce the non-viability of infected cells by 50%. The 50% cytotoxic dose (CD_{50}) was defined as the concentration of compound required to reduce by 50% the number of viable cells in mock-infected cell cultures.

Results

TABLE 2

Anti-HIV Activity of the dImd Nucleoside Analog in Cell Culture

EC_{50} (μM)				CD_{50} (μM)	
HIV-1		HIV-2			
CEM	MT-4	CEM	MT-4	CEM	MT-4
66 ± 20	8.1 ± 1.0	400 ± 0.0	19.5 ± 12	>400	>400

As illustrated above, as little as about 8 μM of dImd has efficacy against HIV. Moreover, since the dImd used in this assay had a free 3'-OH chain termination does not contribute to the inhibitory

activity observed. A chain terminating analog of the present invention therefore would likely be an even more effective anti-HIV agent than a non-chain terminating compound of the present invention.

EXAMPLE 6

INHIBITION OF HIV INFECTION IN CULTURED CELLS WITHOUT CYTOTOXICITY

The representative compound, 1-(2-deoxy-ß-D-ribofuranosyl)-4-acetylimidazolin-2-one (dImd), was also tested for efficacy against HIV by the National Cancer Institute (NCI) using NCI standardized procedures. In this test dImd had a free 3'-OH and yet still exhibited inhibitory activity against HIV with little or no cytotoxicity at concentrations up to 1 mM.

Materials and Methods

dImd with a 2'-deoxy, a 3'-OH and a 5'-OH was prepared as described in Example 1.

The standardized assay performed was a modification of the method of Weislow et al. (1989 J. Natl. Cancer Inst. 81: 577-586) and is designed to detect the effects of anti-HIV agents acting at any stage to the viral reproductive cycle. The assay detects cell killing by HIV and also permits a determination of the amount of anti-HIV agent required to protect cells from cell death.

dImd was dissolved in dimethyl sulfoxide and diluted 1:100 in cell culture medium before serial half-$\log_{10}$ dilutions of dImd were prepared. T4 lymphocytes (CEM cell line) were added and after a brief interval HIV-1 was also added. Uninfected cells were treated with the compound to serve as a toxicity control, while infected and uninfected cells cultured without dImd served as basic controls.

Cultures were incubated at 37° in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT, was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitate formazan production and viewed microscopically for detection of viable cells and confirmation of protective activity. Drug-treated virus-infected cells were compared with drug-treated noninfected cells and with other appropriate controls (untreated infected and untreated noninfected cells, drug-containing wells without cells, etc) on the same plate. Data were reviewed in comparison with other tests done at the same time and a determination of activity was made.

Results

As depicted in Fig. 6 dImd an effective inhibitor of HIV infectivity. Moreover this anti-HIV effect was achieved without chain termination by the present dImd analog since this analog has a free 3'-OH. A chain-terminating analog of the present invention can have greater efficacy against HIV than a non-chain terminating compound of the present invention. Significantly, dImd exhibited no toxicity in concentrations up to 1 mM, the highest concentration tested.

EXAMPLE 7

INHIBITION OF HIV INFECTION IN CULTURED CELLS WITHOUT CYTOTOXICITY

The representative compound, 1-(2-Deoxy-ß-D-ribofuranosyl)-4-acetylimidazolin-2-one (dImd), was also tested for efficacy against HIV in mitogen-stimulated human peripheral blood mononuclear cells (PBMC) infected with HIV-1. In this test dImd had a normal 3'-OH and yet still exhibited inhibitory activity against HIV with little or no cytotoxicity.

Materials and Methods

dImd with a 2'-deoxy, a 3'-OH and a 5'-OH was prepared as described in Example 1.

The assay was performed as described by Bardos et al. (1992 Antimicrob. Agents and Chemother. 36: 108-114).

Mitogen-stimulated human PBMC were infected with HIV-1 (strain LAV), Schinazi et al. (1988 Antimicrob. Agents Chemother., 32: 1784-1787). The virus concentration used for infection was about 63,000 dpm of reverse transcriptase (RT) activity per 10^7 cells per 10 ml of medium. Analog dImd was added about 45 min. after infection. Cultures were maintained in a humidified 5% CO_2-95% air incubator at 37°C for 6 days after infection, at which point all cultures were sampled for supernatant RT activity. Previous studies by Schinazi and coworkers had indicated that maximum RT levels were obtained at that time, Chu et al. (1989 J. Med. Chem. 32: 612-617); Chu et al. (1988 Biochem. Pharmacol. 37: 3543-3548); Lin et al. (1988 J. Med.

Chem. 31: 336-340); Schinazi et al. (1990 Antimicrob. Agents Chemother. 34: 1061-1067). The supernatant was clarified, and the virus particles were pelleted at 100,000 x g for 30 min by using a 70.1 Ti rotor (Beckman Instruments, Fullerton, Calif.) and suspended in virus-disrupting buffer. The RT assay was performed in 96-well microdilution plates using poly(rA)$_n$ (dT)$_{12-18}$ as template-primer in the method of Schinazi et al. (1988 Antimicrob. Agents Chemother., 32: 1784-1787).

dImd was also evaluated for toxic effects on uninfected phytohemagglutinin-stimulated human PBMC using a radioactive thymidine uptake method. Briefly, cells in a 96-well plate were grown in the presence of drug for 24 h, and then 1 µCi of [^{3}H]thymidine (specific activity, 69 Ci/mmol) was added to each well. After 24 h, the cells were harvested on glass fibers, washed, and dried, and the amount of radioactivity associated with the cells was determined. Cycloheximide was included as a control for toxicity in every assay.

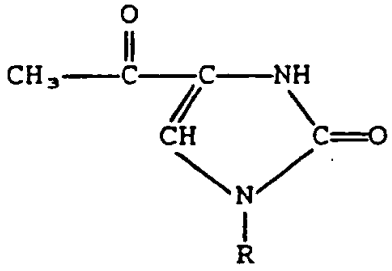
Results

The effective concentration of dImd for 50% inhibition of HIV-1 reverse transcriptase (EC_{50}) by viable virus particles was 8.4 µM. In contrast no cytotoxicity was observed for cultured PBMC, CEM and Vero cells treated with up to 100 µM dImd. Therefore, dImd, even with a free 3'-OH, is a highly selective inhibitor of HIV replication. A chain-terminating analog of the present invention would be expected to have greater efficacy against HIV than a non-chain terminating compound of the present invention.

WHAT IS CLAIMED:

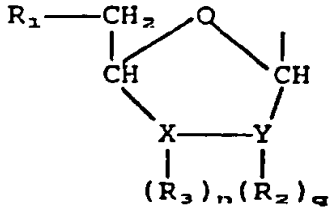
1. A nucleoside or a nucleotide compound comprising a 4-acetylimidazolin-2-one base.

2. A compound of the formula:

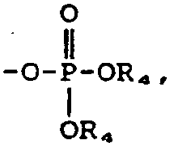
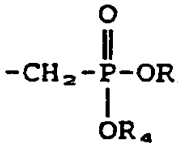
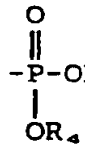


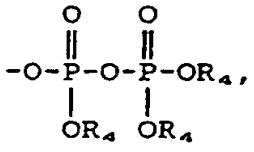
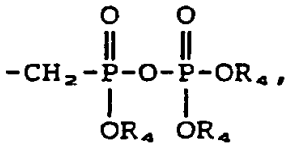
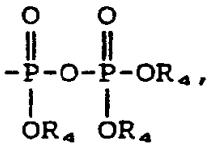
I

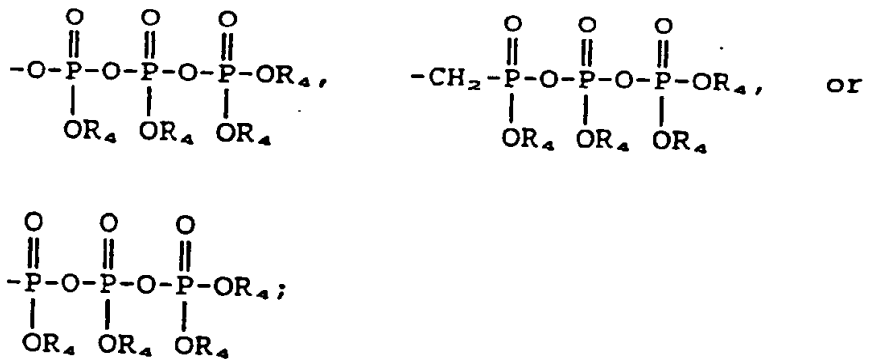
wherein R is hydrogen or



wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate,  ,  ,  ,

 ,  ,  ,



or

R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

X and Y each are independently -CH-, -O-, -S-, or X and Y together are -C=C-;

R_2 is hydrogen, lower alkoxy or hydroxy;

R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido;

n and q are independently 0 or 1;

when X is -O- or -S- then n is zero;

when Y is -O- or -S- then q is zero; or a pharmaceutically acceptable salt thereof.

3. The compound of Claim 2 wherein n is 1.

4. The compound of Claim 3 wherein R_3 is hydrogen, hydroxy, halo or azido.

5. The compound of Claim 4 wherein said halo is fluoro.

6. The compound of Claim 3 wherein X is -CH or -O-.

7. The compound of Claim 3 wherein X and Y together are -C=C-.

8. The compound of Claim 3 wherein q is 1.

9. The compound of Claim 8 wherein Y is CH.

10. The compound of Claim 8 wherein X and Y together are -C=C-.

11. The compound of Claim 8 wherein R_2 is hydrogen.

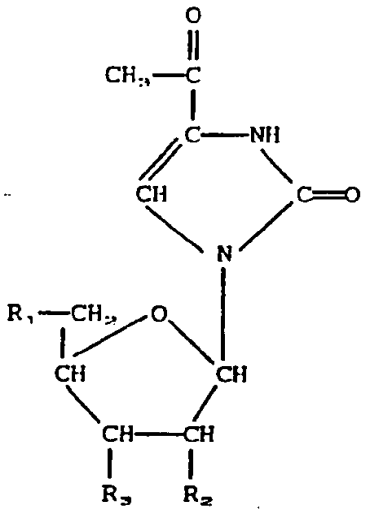
12. The compound of Claim 2 wherein n is 0.

13. The compound of Claim 12 wherein X is -O-.

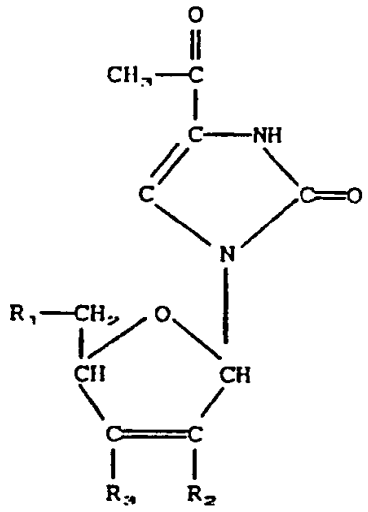
14. The compound of Claim 2 wherein q is 0.

15. The compound of Claim 14 wherein Y is -O-.

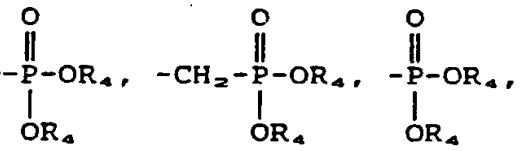
16. A compound of the formula:

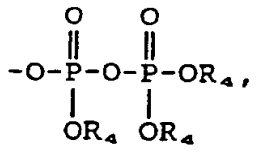
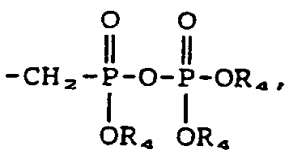
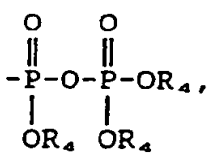


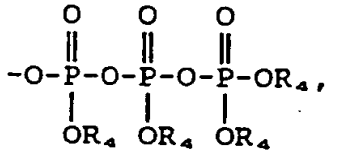
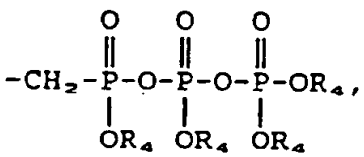
or

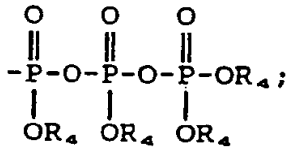


wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, 

1  ,  ,  ,

5  ,  or

10  ;

15 R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

R_2 is hydrogen, lower alkoxy or hydroxy;

R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido; or

20 a pharmaceutically acceptable salt thereof.

17. The compound of Claim 16 wherein R_3 is hydrogen, hydroxy, halo or azido.

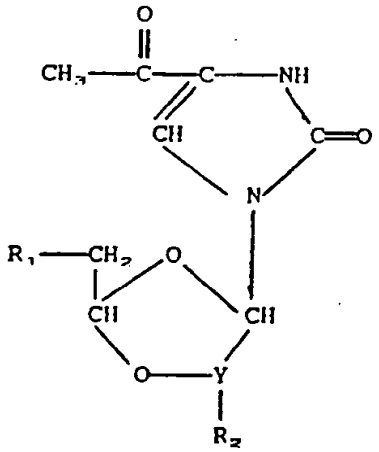
18. The compound of Claim 17 wherein said halo is fluoro.

25 19. The compound of Claim 17 wherein R_2 is hydrogen.

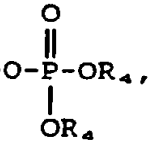
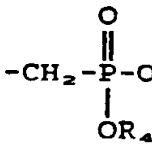
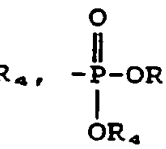
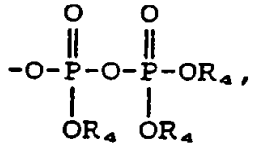
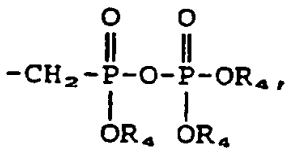
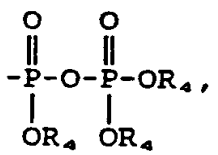
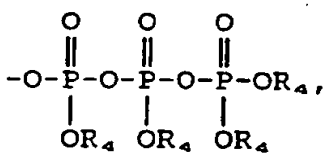
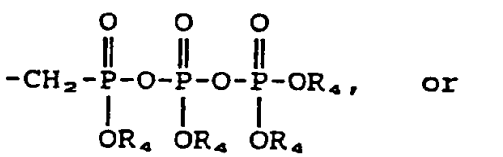
30

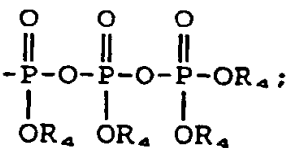
35

20. A compound of the formula:



wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate,  ,  ,  ,  ,  ,  ,  ,  , or



R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

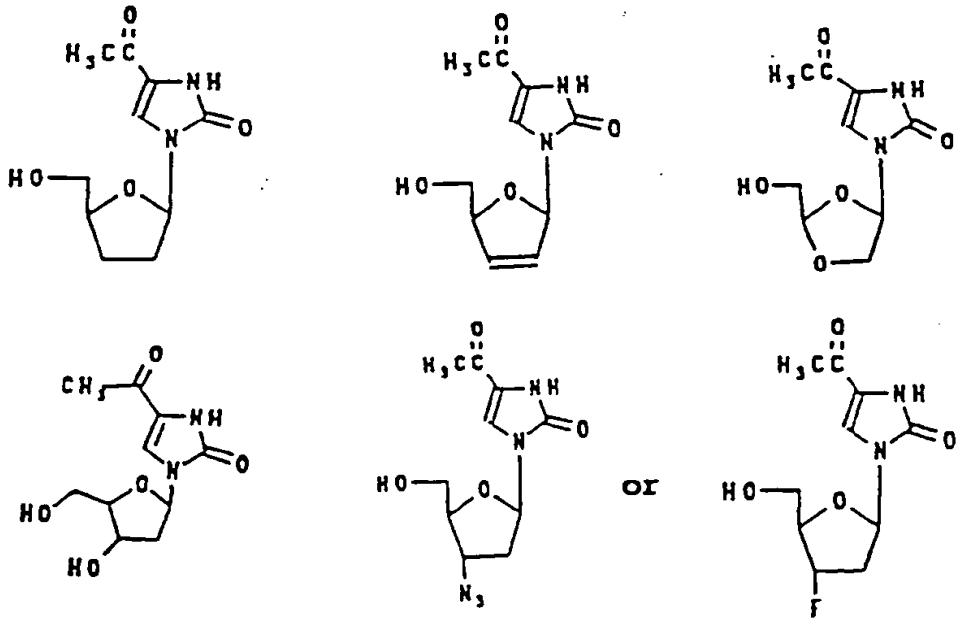
R_2 is hydrogen, lower alkoxy or hydroxy; or a pharmaceutically acceptable salt thereof.

21. The compound of Claim 20 wherein R_2 is hydrogen.

22. The compound of any one Claims 2, 16 or 20 wherein R_1 is -OH, $-O-PO(OR_4)_2$, $-CH_2-O-PO-(OR_4)_2$, $-PO(OR_4)_2$ or $-O-PO(OR_4)-O-PO(OR_4)-O-PO(OR_4)_2$.

23. The compound of any one Claims 2, 16 or 20 wherein said pharmaceutically acceptable salt is a sodium, potassium, lithium, calcium, magnesium, barium, ammonium, monoethanolammonium or tri-(cycolhexylammonium) salt.

24. A compound of the formulae:



25. A pharmaceutical composition comprising a pharmaceutically effective amount of the compound of any one of Claims 1, 2, 16, 20 or 24 and a physiologically acceptable carrier.

26. The pharmaceutical composition of Claim 25 wherein said pharmaceutically effective amount is an anti-viral effective amount, a reverse transcriptase-inhibiting amount, a retrovirus replication-inhibiting amount, a hepatitis B replication-inhibiting amount or a human immunodeficiency virus-inhibiting amount.

27. The pharmaceutical composition of Claim 25 wherein said pharmaceutically effective amount is sufficient to provide about 0.001 mg/kg/day to about 500 mg/kg/day.

28. The pharmaceutical composition of Claim 25 wherein said pharmaceutically effective amount is about 0.01 mg to about 1 g in unit dosage form.

29. The pharmaceutical composition of Claim 25 for administration by oral, topical, intradermal, intraveneous, intramuscular, intraperitoneal or subcutaneous delivery.

30. A method of inhibiting DNA synthesis catalyzed by reverse transcriptase which comprises contacting said reverse transcriptase with at least one nucleoside analog or a nucleotide analog comprising a 4-acetylimidazolin-2-one base in an amount sufficient to inhibit reverse transcriptase-catalyzed DNA synthesis.

31. The method of Claim 30 wherein said amount does not substantially inhibit DNA synthesis catalyzed by human nuclear DNA polymerase.

32. The method of Claim 30 wherein said amount inhibits reverse transcriptase DNA synthesis by about 50% to about 80%.

33. A method of inhibiting viral replication mediated by reverse transcriptase which comprises contacting a virus whose replication involves DNA synthesis catalyzed by reverse transcriptase with at least one nucleoside analog or at least one nucleotide analog comprising a 4-acetylimidazolin-2-one base in an amount sufficient to inhibit viral replication.

34. The method of Claim 33 wherein said amount is not cytotoxic for mammalian cells.

35. The method of Claim 33 wherein said amount inhibits virus replication by about 50% to about 80%.

36. The method of Claim 33 wherein said virus is a lentivirus, oncovirus C or hepatitis B virus.

37. The method of Claim 33 wherein said virus is human immunodeficiency virus-1, human immunodeficiency virus-2, human T cell leukemia/lymphoma virus type I, human T cell leukemia/lymphoma virus type II, hepatitis B virus, feline immunodeficiency virus, simian immuno-deficiency virus, visna virus of sheep, caprine arthritis-encephalitis virus or equine infectious anemia virus.

38. The method of Claim 33 wherein said virus is human immunodeficiency virus-1, human immunodeficiency virus-2, human T cell leukemia/lymphoma virus type I, human T cell leukemia/lymphoma virus type II or hepatitis B virus.

39. A method of treating or preventing animal retroviral infection which comprises administering to an

animal an anti-retroviral effective amount of at least one nucleoside analog or at least one nucleotide analog comprising a 4-acetylimidazolin-2-one base.

40. The method of Claim 39 wherein said amount does not substantially inhibit DNA synthesis catalyzed by a human nuclear DNA polymerase.

41. The method of Claim 39 wherein said amount inhibits retrovirus replication by about 50% to about 80%.

42. The method of Claim 39 wherein said retroviral infection is caused by a lentivirus or an oncovirus C.

43. A method of treating or preventing hepatitis B infection which comprises administering to a patient an anti-hepatitis B effective amount of at least one nucleoside analog or at least nucleotide analog comprising a 4-acetylimidazolin-2-one base.

44. The method of Claim 43 wherein said anti-hepatitis B effective amount does not substantially inhibit DNA synthesis catalyzed by a human nuclear DNA polymerase.

45. The method of Claim 43 wherein said anti-hepatitis B effective amount inhibits hepatitis B replication by about 50% to about 80%.

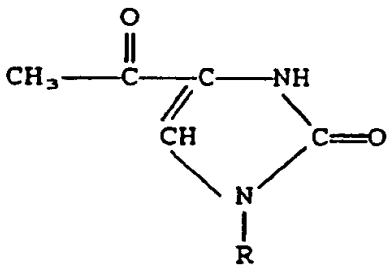
46. A method of treating or preventing human immunodeficiency virus (HIV) infection which comprises administering to a patient an anti-HIV effective amount of at least one nucleoside or nucleotide analog comprising a 4-acetylimidazolin-2-one base.

47. The method of Claim 46 wherein said anti-HIV effective amount does not substantially inhibit DNA synthesis catalyzed by a human nuclear DNA polymerase.

48. The method of Claim 46 wherein said anti-HIV effective amount inhibits HIV replication by about 50% to about 80%.

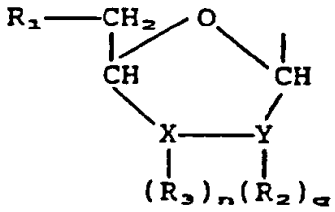
49. The method of Claim 46 wherein said human immunodeficiency virus infection is caused by human immunodeficiency virus-1 or human immunodeficiency virus-2.

50. The method of any one of Claims 30, 33, 39, 43 or 46 wherein said nucleoside analog or said nucleotide analog comprises a compound of the formula:



I

wherein R is:



wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, $-O-P(=O)(OR_4)-OR_4$, $-CH_2-P(=O)(OR_4)-OR_4$, $-P(=O)(OR_4)-OR_4$,

$-O-P(=O)(OR_4)-O-P(=O)(OR_4)-OR_4$, $-CH_2-P(=O)(OR_4)-O-P(=O)(OR_4)-OR_4$, $-P(=O)(OR_4)-O-P(=O)(OR_4)-OR_4$,

$-O-P(=O)(OR_4)-O-P(=O)(OR_4)-O-P(=O)(OR_4)-OR_4$, $-CH_2-P(=O)(OR_4)-O-P(=O)(OR_4)-O-P(=O)(OR_4)-OR_4$, or

$-P(=O)(OR_4)-O-P(=O)(OR_4)-O-P(=O)(OR_4)-OR_4$;

R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

X and Y each are independently $-\underset{|}{CH}-$, -O-, -S-, or X and Y together are -C=C-;

R_2 is hydrogen, lower alkoxy or hydroxy;

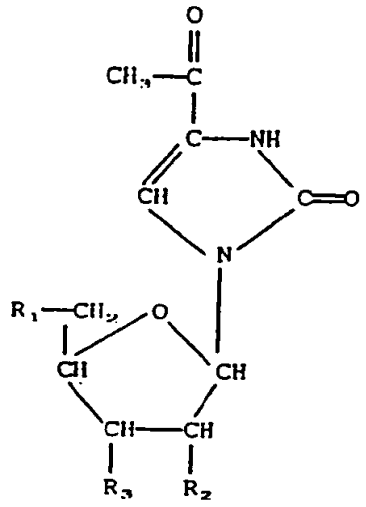
R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido;

n and q are independently 0 or 1;

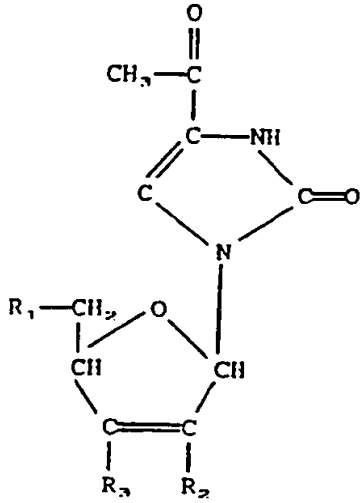
when X is -O- or -S- then n is zero;

when Y is -O- or -S- then q is zero; or a pharmaceutically acceptable salt thereof.

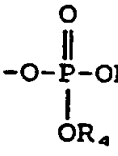
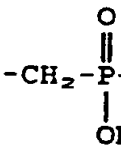
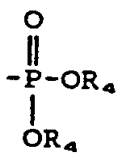
51. The method of Claim 50 wherein said compound is of the formula:

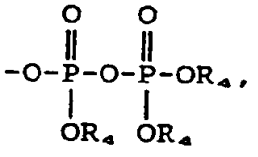
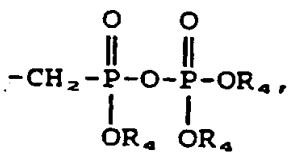
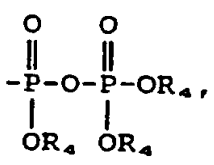


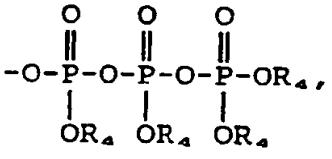
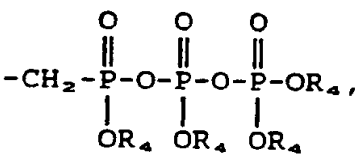
or

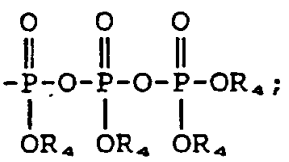


wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate,  ,  ,  ,

 ,  ,  ,

 ,  , or



R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

R_2 is hydrogen, lower alkoxy or hydroxy;

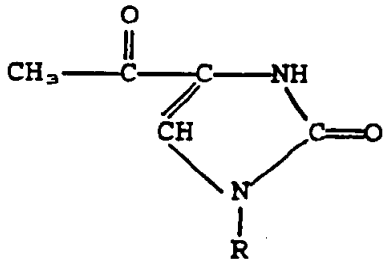
R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido; or a pharmaceutically acceptable salt thereof.

AMENDED CLAIMS

[received by the International Bureau on 28 February 1994 (28.02.94); original claims 1 and 2 unchanged; original claims 3 - 51 replaced by amended claims 3 - 26 (11 pages)]

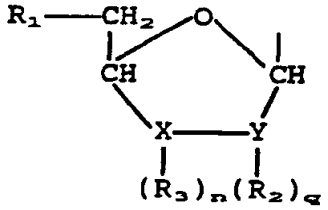
1. A nucleoside or a nucleotide compound comprising a 4-acetylimidazolin-2-one base.

2. A compound of the formula:

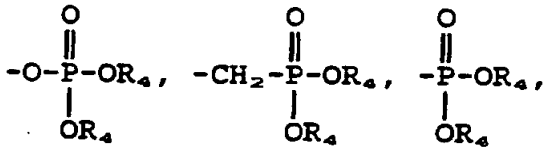


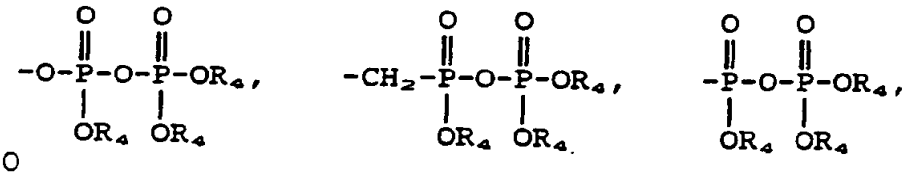
I

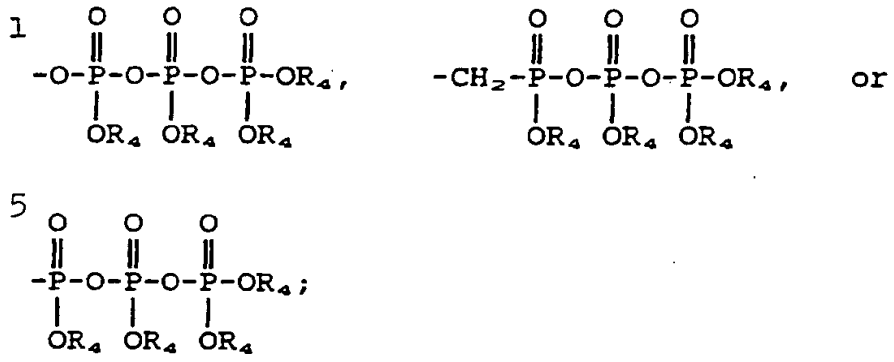
wherein R is hydrogen or



wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, 





or

R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

X and Y each are independently -CH-, -O-, -S-, or X and Y together are -C=C-;

R_2 is hydrogen, lower alkoxy or hydroxy;

R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido;

n and q are independently 0 or 1;

when X is -O- or -S- then n is zero;

when Y is -O- or -S- then q is zero; or

a pharmaceutically acceptable salt thereof.

3. The compound of Claim 2 wherein n is 1, R_3 is hydrogen, hydroxy, halo or azido, X is -CH or -O-, q is 1, Y is CH and R_2 is hydrogen.

4. The compound of Claims 2 or 3 wherein said halo is fluoro.

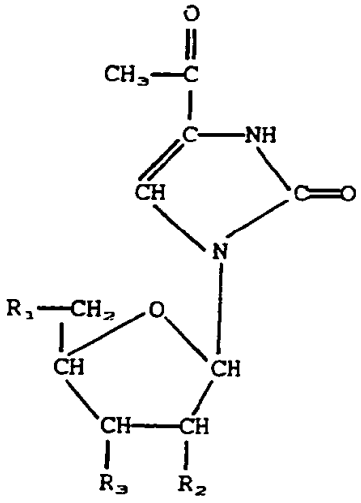
5. The compound of any of Claims 2-4 wherein X and Y together are -C=C-.

6. The compound of Claim 2 wherein n is 0 and X is -O-.

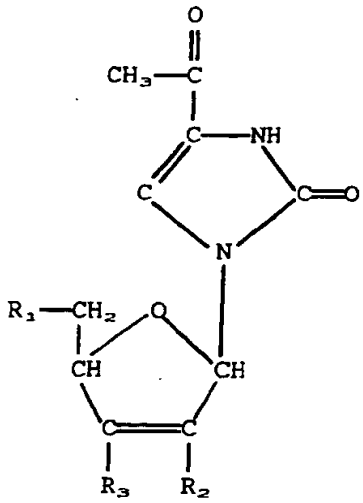
7. The compound of Claim 2 wherein q is 0 and

Y is -O-.

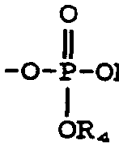
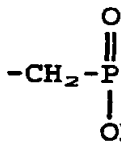
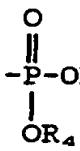
8. A compound of the formula:

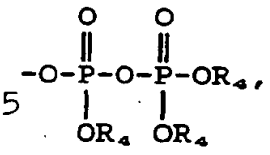
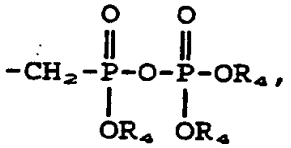
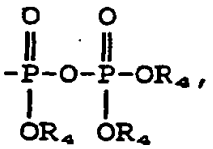


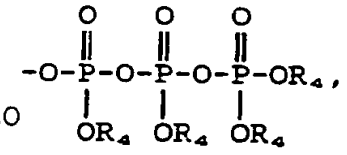
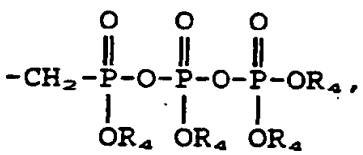
or



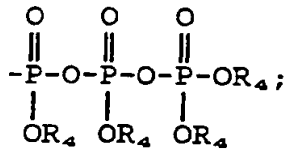
wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate,  ,  ,  ,

 ,  ,  ,

 ,  or

AMENDED SHEET (ARTICLE 19)



R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

R_2 is hydrogen, lower alkoxy or hydroxy;

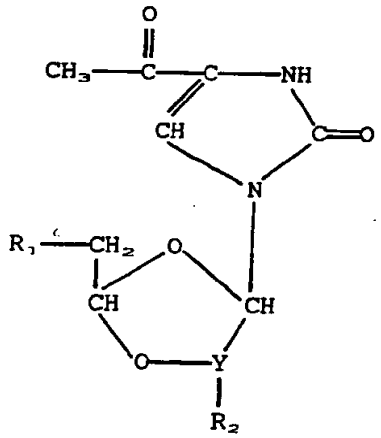
R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido; or

a pharmaceutically acceptable salt thereof.

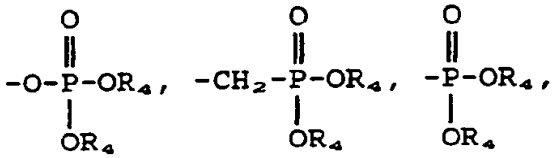
9. The compound of Claim 8 wherein R_3 is hydrogen, hydroxy, halo or azido and R_2 is hydrogen.

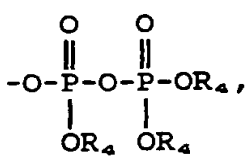
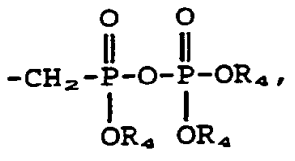
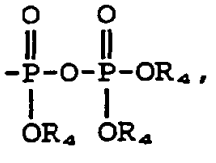
10. The compound of Claims 8 or 9 wherein said halo is fluoro.

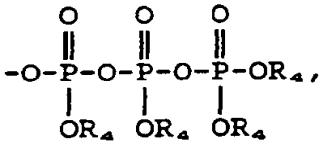
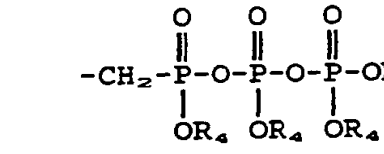
11. A compound of the formula:

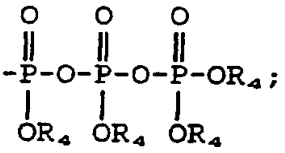


wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, 



or



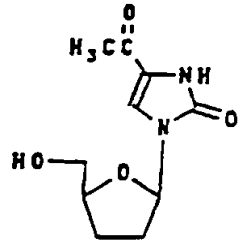
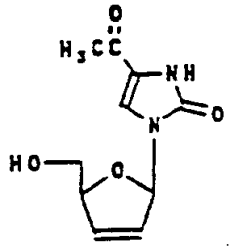
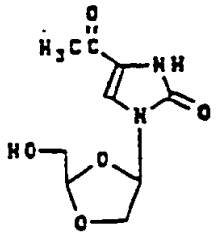
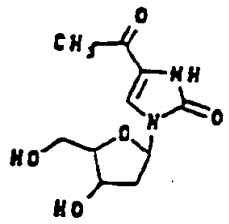
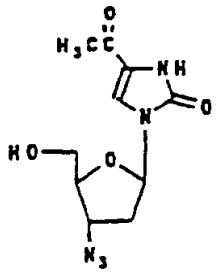
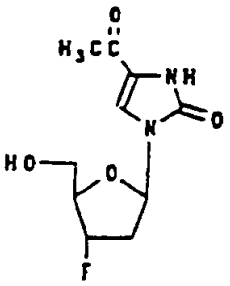
R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

R_2 is hydrogen, lower alkoxy or hydroxy; or a pharmaceutically acceptable salt thereof.

12. The compound of any of Claims 2-11 wherein R_1 is -OH, $-O-PO(OR_4)_2$, $-CH_2-O-PO-(OR_4)_2$, $-PO(OR_4)_2$ or $-O-PO(OR_4)-O-PO(OR_4)-O-PO(OR_4)_2$.

13. The compound of any of Claims 2-12 wherein said pharmaceutically acceptable salt is a sodium, potassium, lithium, calcium, magnesium, barium, ammonium, monoethanolammonium or tri-(cycolhexylammonium) salt.

14. A compound of the formulae:






or


15. A pharmaceutical composition comprising a pharmaceutically effective amount of the compound of any of Claims 1-14 and a physiologically acceptable carrier.

16. The pharmaceutical composition of Claim 15 wherein said pharmaceutically effective amount is sufficient to provide about 0.001 mg/kg/day to about 500 mg/kg/day and is about 0.01 mg to about 1 g in unit dosage form.

17. A method of inhibiting DNA synthesis catalyzed by reverse transcriptase or viral replication mediated by reverse transcriptase which comprises contacting said reverse transcriptase or virus whose replication involves DNA synthesis catalyzed by reverse transcriptase with at least one nucleoside analog or a nucleotide analog comprising a 4-acetylimidazolin-2-one base in an amount sufficient to inhibit reverse transcriptase-catalyzed DNA synthesis or viral replication.

18. The method of Claim 17 wherein said amount inhibits reverse transcriptase DNA synthesis or viral replication by about 50% to about 80%.

19. The method of Claim 33 wherein said amount is not cytotoxic for mammalian cells.

20. The method of any of Claims 17-19 wherein said virus is a lentivirus, oncovirus C or hepatitis B virus, human immunodeficiency virus-1, human immunodeficiency virus-2, human T cell leukemia/lymphoma virus type I, human T cell leukemia/lymphoma virus type II, hepatitis B virus, feline immunodeficiency virus, simian immuno-deficiency virus, visna virus of sheep, caprine arthritis-encephalitis virus or equine infectious anemia virus.

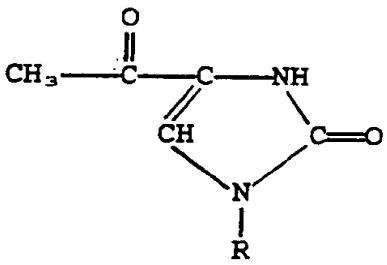
21. A method of treating or preventing animal retroviral infection which comprises administering to an animal an anti-retroviral effective amount of at least one nucleoside analog or at least one nucleotide analog comprising a 4-acetylimidazolin-2-one base.

22. The method of Claim 21 wherein said amount inhibits retrovirus replication by about 50% to about 80%.

23. A method of treating or preventing hepatitis B infection or human immunodeficiency virus (HIV) infection which comprises administering to a patient an anti-hepatitis B or anti-HIV effective amount of at least one nucleoside analog or at least nucleotide analog comprising a 4-acetylimidazolin-2-one base.

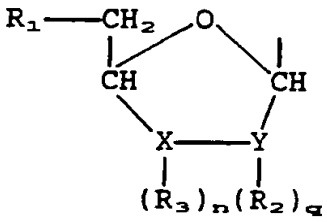
24. The method of Claim 23 wherein said anti-hepatitis B or anti-HIV effective amount inhibits hepatitis B or HIV replication by about 50% to about 80%.

25. The method of any of Claims 17-24 wherein said nucleoside analog or said nucleotide analog comprises a compound of the formula:

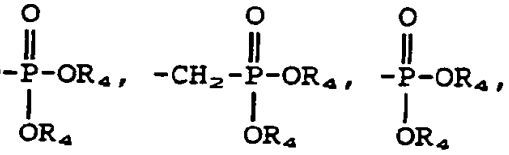


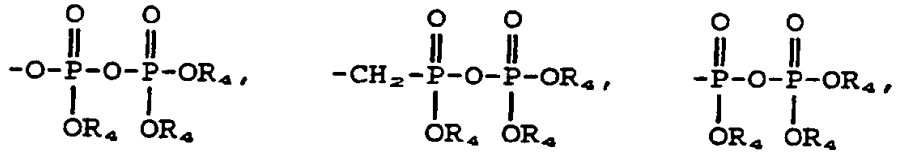
I

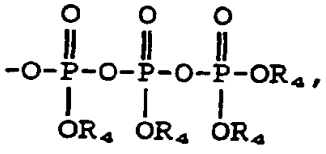
wherein R is:

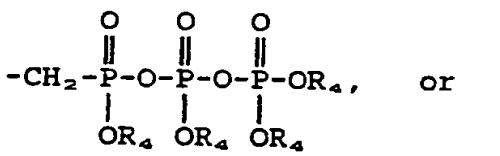


wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, 







R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

X and Y each are independently -CH-, -O-, -S-, or X and Y together are -C=C-;

R_2 is hydrogen, lower alkoxy or hydroxy;

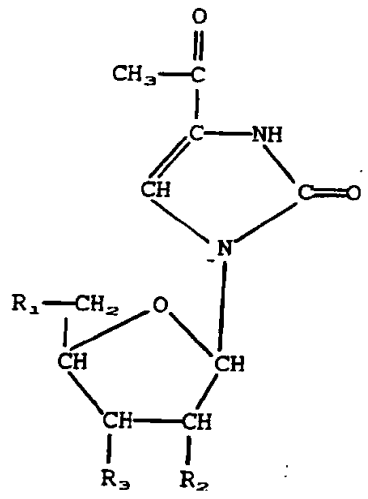
R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido;

n and q are independently 0 or 1;

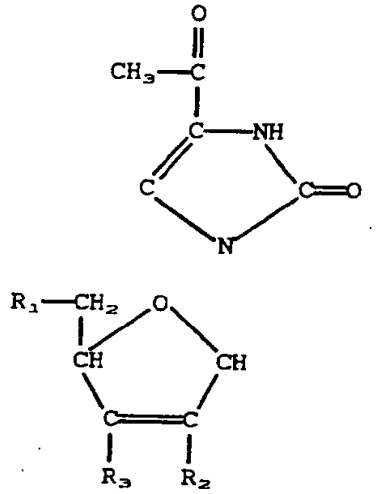
when X is -O- or -S- then n is zero;

when Y is -O- or -S- then q is zero; or a pharmaceutically acceptable salt thereof.

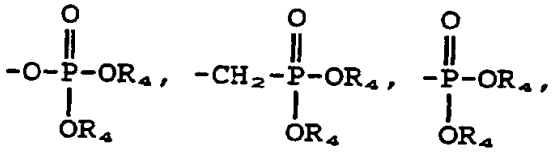
26. The method of Claim 25 wherein said compound is of the formula:

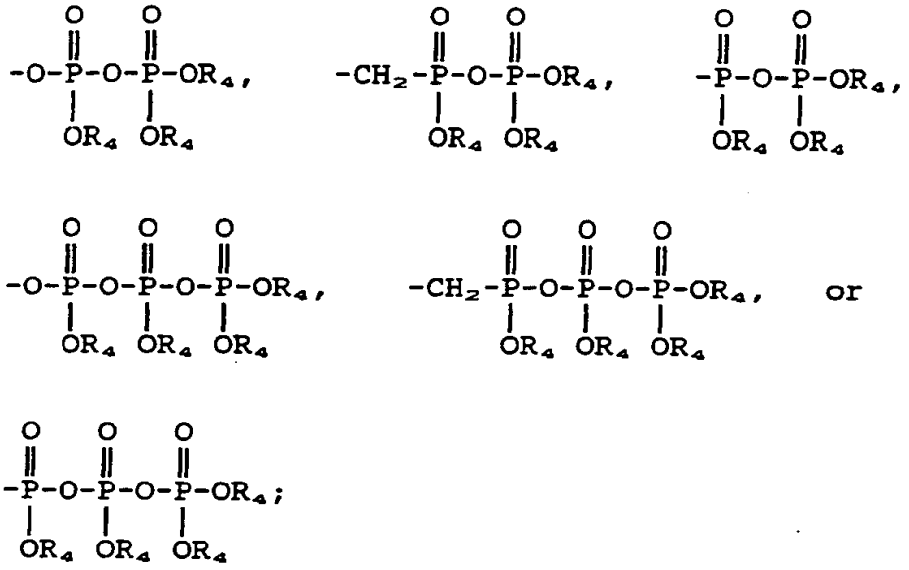


or



wherein:

R_1 is hydroxy, monophosphate, diphosphate, triphosphate, phosphonate, 

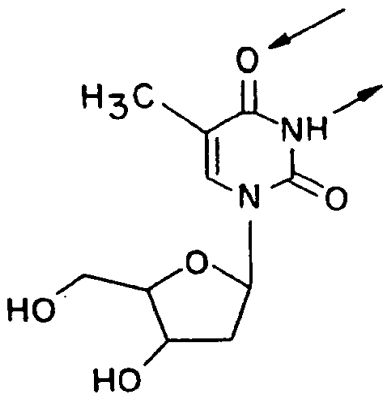


R_4 is hydrogen, cation, lower alkyl or acyloxymethyl;

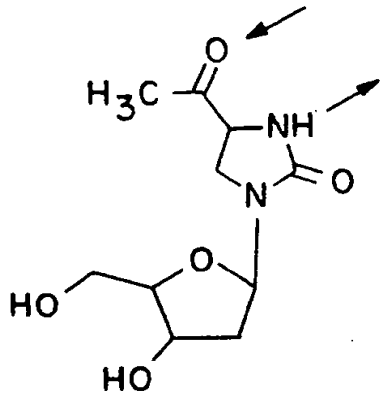
R_2 is hydrogen, lower alkoxy or hydroxy;

R_3 is hydrogen, lower alkoxy, hydroxy, halo, azido; or a pharmaceutically acceptable salt thereof.

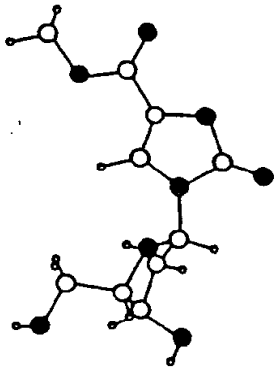
FIG. 1



THYMIDINE



IMIDINE



4-METHOXYCARBONYL DERIVATIVE

FIG. 2

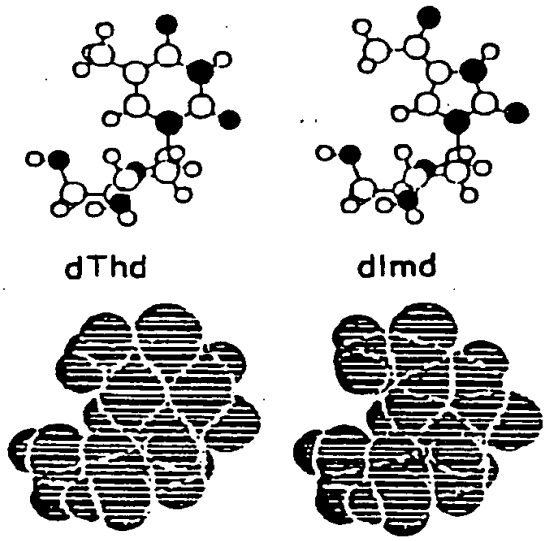
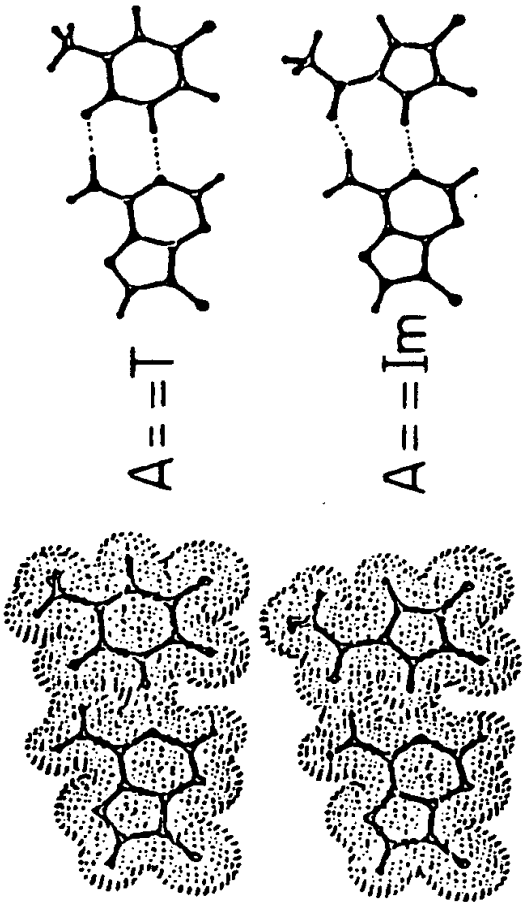


FIG. 3



	N-H...O Å	N...H-N Å	<N-H...O degrees	<N...H-N degrees
A = = T	2.804	2.954	173.42	178.50
A = = Im	2.614	3.060	160.26	162.86

FIG. 4

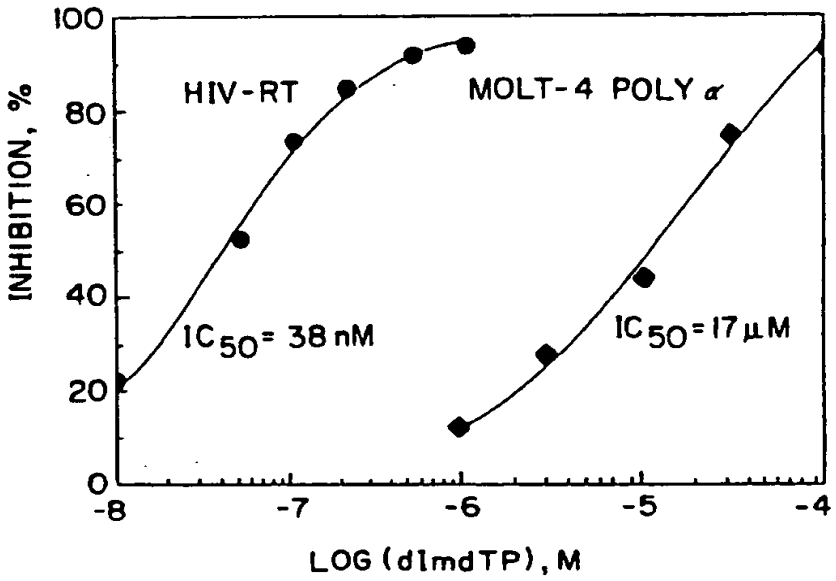


FIG. 5

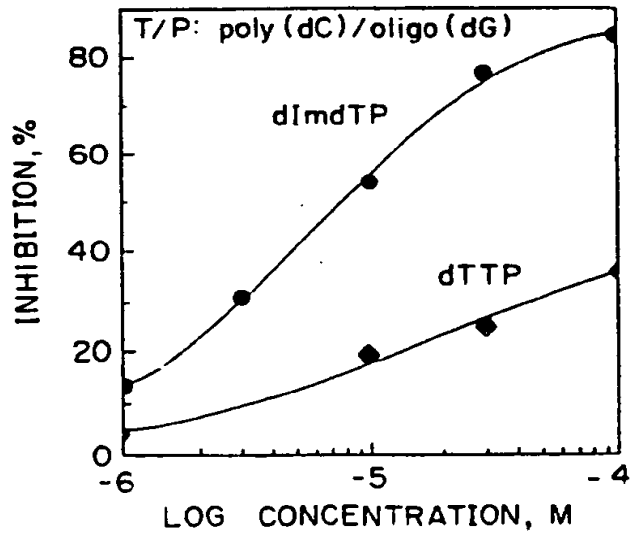
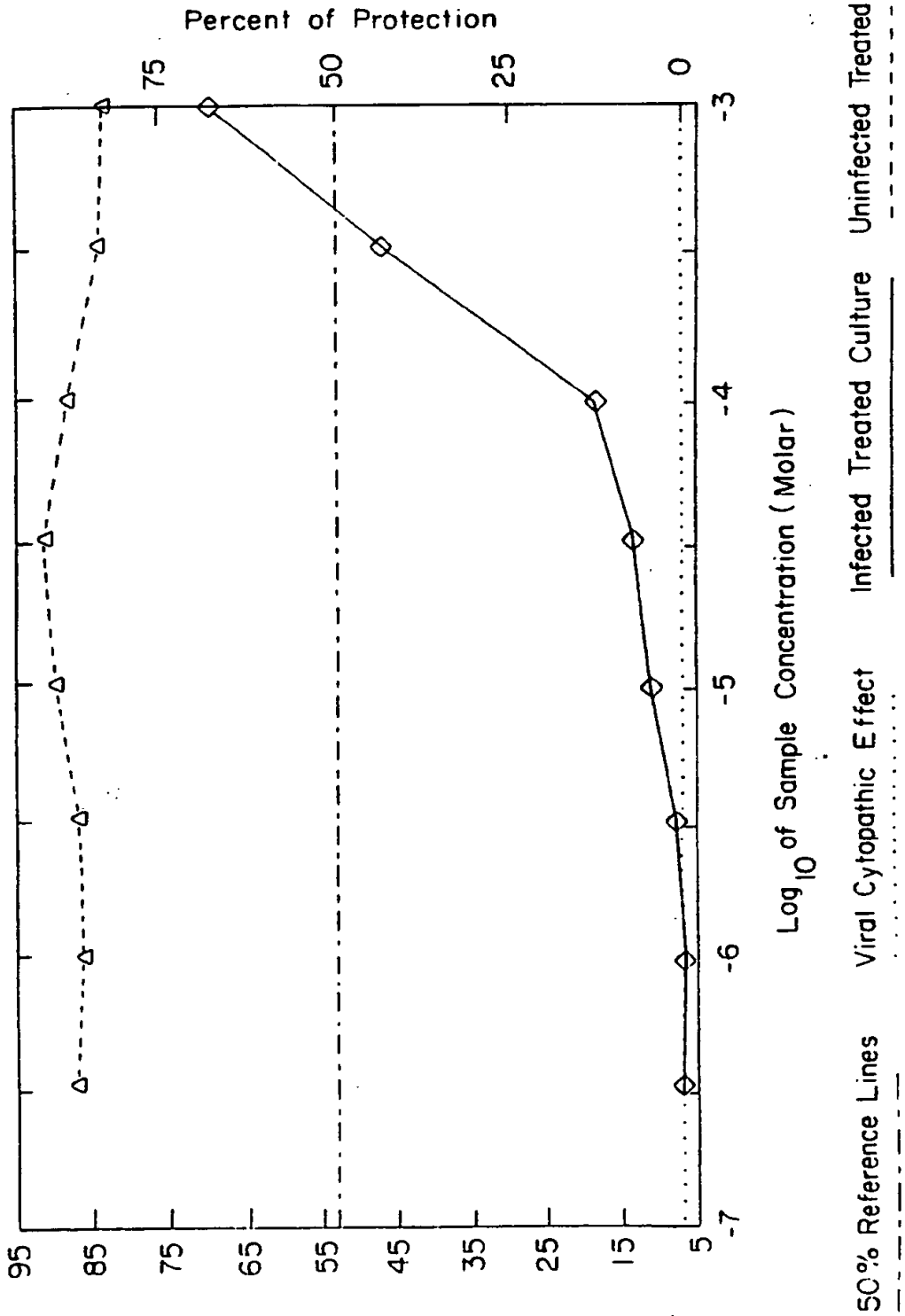


FIG. 6



INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/US 93/02472

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07H19/052 C07F9/6558 C07D405/04 A61K31/70 A61K31/66
A61K31/415 C07D233/70 C07D411/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07H C07F C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US,A,2 441 933 (R. DUSCHINSKY) 30 July 1945 see page 1, column 2; claim 1 ---	2
X	EP,A,0 079 049 (MERREL DOW PHARMACEUTICALS INC.) 18 May 1983 see claim 1 ---	2
X	TETRAHEDRON LETTERS vol. 25, no. 28 , 10 August 1984 , OXFORD, UK pages 2957 - 2960 J. L. LAMATTINA ET AL 'The reaction of 5-acetyl-2-aminooxazole with amines: an approach to 1H-5-acetyl-2-aminoimidazoles' see page 2959, line 11 - line 12 --- -/--	2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 December 1993	30. 12. 93

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Moreno, C

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 06102 (MEDIVIR AB) 16 April 1992 see the whole document	1,2,43, 46
A	CHEMICAL ABSTRACTS, vol. 103, no. 25, 23 December 1985, Columbus, Ohio, US; abstract no. 215736a, 'Imidazole nucleoside derivatives' page 939 ;column 1 ; see abstract & JP,A,60 109 595 (YAMANOUCHI PHARMACEUTICAL CO.)	1,2
A	CHEMICAL ABSTRACTS, vol. 103, no. 25, 23 December 1985, Columbus, Ohio, US; abstract no. 215737b, '3-Deazaguanosine derivatives' page 939 ;column 1 ; see abstract & JP,A,60 109 594 (YAMASA SHOYU CO. LTD.)	1,2

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/US 93/02472

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
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EP-A-0079049	18-05-83	US-A-	4367236	04-01-83
		AU-B-	555244	18-09-86
		AU-A-	9011382	12-05-83
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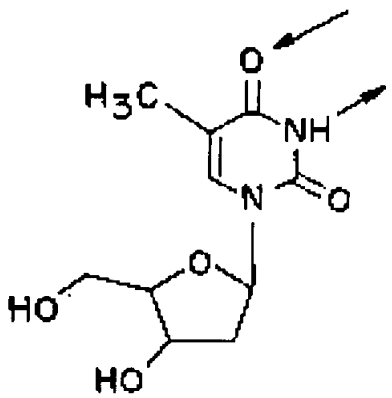
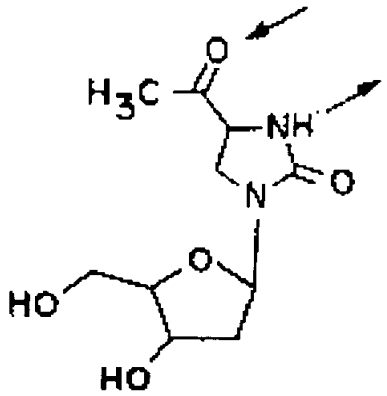
		EP-A-	0554274	11-08-93
JP-A-60109595	15-06-85	NONE		
JP-A-60109594	15-06-85	NONE		

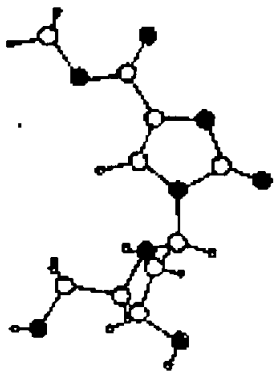
FIG. 1



THYMIDINE



IMIDINE



4-METHOXYCARBONYL DERIVATIVE

FIG. 2

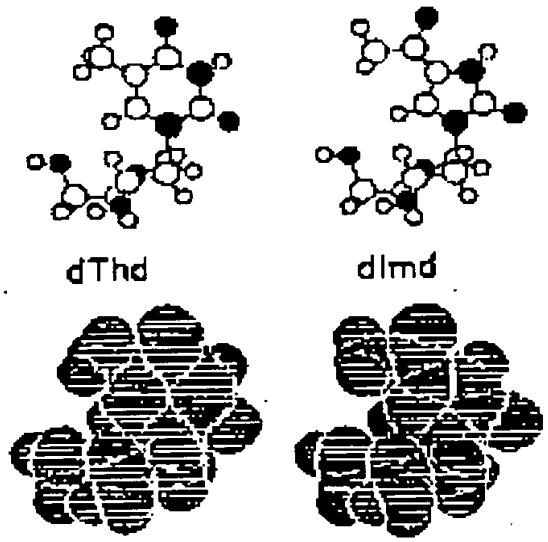
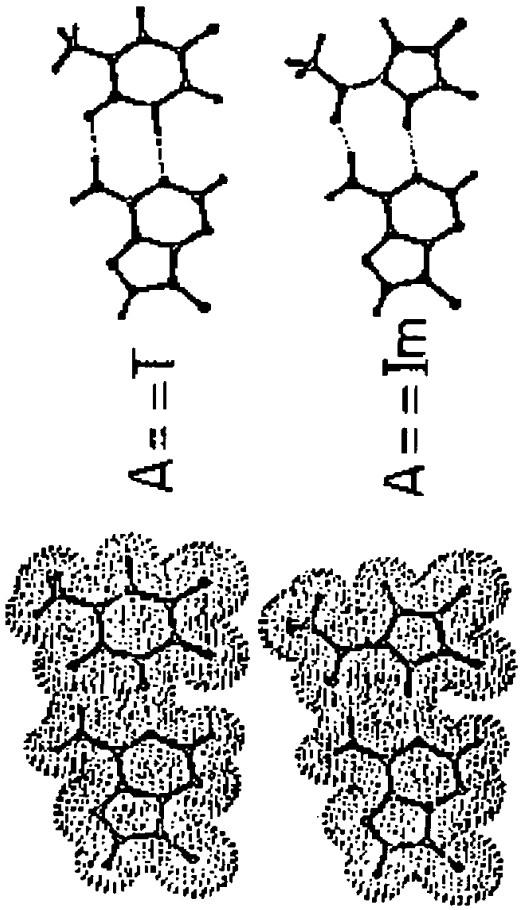


FIG. 3



	N-H...O Å	N...H-N Å	<NH...O degrees	<N...H-N degrees
A=T	2.804	2.954	173.42	178.50
A=Im	2.614	3.060	160.26	162.86

FIG. 4

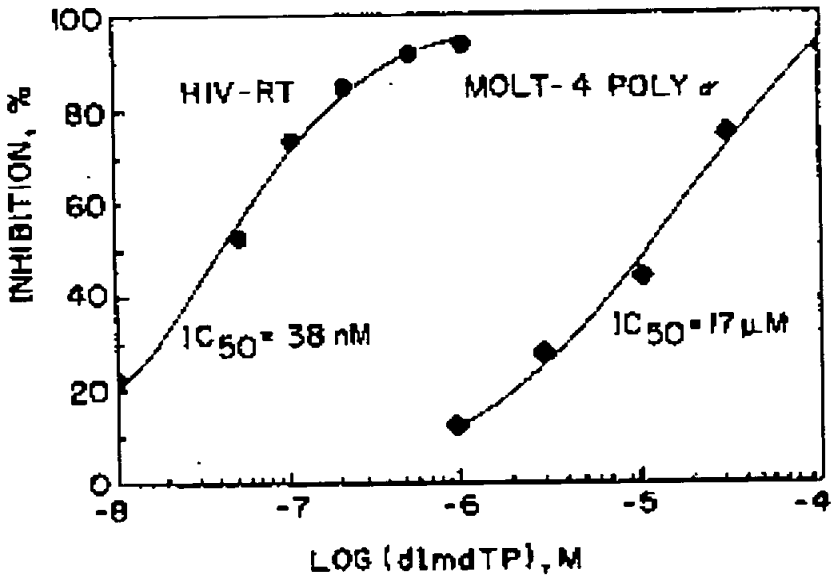


FIG. 5

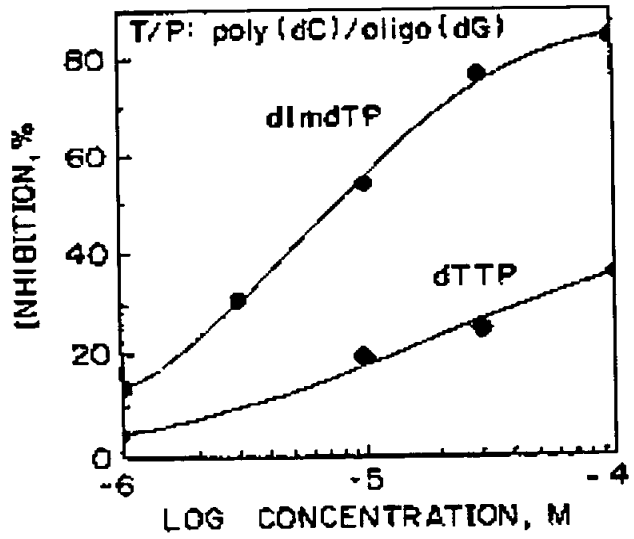


FIG. 6